AMYLASE AND PANCREATITIS

A CLINICAL AND EXPERIMENTAL STUDY OF THE LEVEL OF AMYLASE IN THE BLOOD SERUM AS A PARAMETER OF ACUTE DISORDERS OF THE PANCREAS.

by

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Chambers Dictionary defines as "the constant quantity which enters into the equation of a curve."

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CONTENTS

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Netherton, 1948.

INTRODUCTION

[illegible] the resemblance between the two organs regarding the presence of an abundant salivary gland. The early studies of pancreatitis were influenced by epidemiological findings in the occasional fatal [illegible] of mumps. Netherton (1948) also refers to a publication which dates from "Hippocrates to the south and whenever."

Netherton's early idea probable. [illegible] he could not have perceived of intrapancreatic salivary duct a place in the pathology of pancreatitis nor could they have known that, nearly a century later, the spilling of bile and wine of excessive amounts of alcohol [illegible] pancreas would be put forward as a diagnostic [illegible]

"Death might take place within a few hours from a fatal metastasis of the buccal salivation to the pancreas."

Neumann, 1836.

Early anatomists (de Graaf, 1671), recognising the resemblance between the two organs, regarded the pancreas as an abdominal salivary gland. The toxic effects of pancreatitis were attributed to excess pancreatic salivation, as in the quotation from Neumann given above. Schmitt (1818) also refers to a patient who died from "ptyalism of the mouth and abdomen."

Neumann's words seem prophetic. He and his contemporaries could not have conceived of intrapancreatic enzymes having a place in the pathology of pancreatitis nor could they have known that, nearly a century later, the spilling into the blood and urine of excessive amounts of diastase from the pancreas would be put forward as a diagnostic feature of acute pancreatitis.

Diastase in human pancreatic juice was not demonstrated until 1845 (Bouchardat and Sandras) and was observed in the blood the following year by Magendie, though their possible common source was not recognised until a good deal later. Lepine and Barral (1891) showed that, in the dog, ligation of the pancreatic ducts produced a marked rise in the serum amylase. Schlesinger (1908) also found that the blood amylase in the dog was markedly reduced after pancreatectomy and he postulated that the pancreas was the source of the amylase circulating in the blood. Wohlgemuth (1908) confirmed both these findings using an improved laboratory technique.

Later, in 1929, Wohlgemuth reported finding the amylase concentration of the blood to be increased in patients with acute pancreatic disease. McCaughan (1934) came to the conclusion that serum amylase estimations might be valuable in the diagnosis of pancreatitis although, as the hyperamylasaemia in this condition was temporary, the value of the test as a routine clinical investigation would be greatest in cases seen in the early stages of the disease.

Progress was made when Somogyi (1938) developed a simple and rapid method for measuring serum amylase and, from early reports by Somogyi and others, it seemed that this biochemical test would become established as a routine procedure of some value in the diagnosis of the acute abdomen. Further experiences showed that hyperamylasaemia was not a specific response limited to primary pancreatic disease and that the test sometimes failed just in those conditions in which it was most needed. Wilson and Seabrook (1953) claimed that in their experience a high serum amylase was not due to pancreatic disease in the majority of cases and they considered that this test was too unreliable to be useful or safe.

The role of surgery in the early stages of acute pancreatitis is essentially diagnostic and most would agree that surgery would be best avoided if diagnosis could otherwise be established. This point was made in the first monograph written upon acute pancreatitis, when Fitz (1889) said: "It (acute pancreatitis) has been repeatedly confounded with acute intestinal obstruction, and thus has led, in several instances, to an ineffective laparotomy - an operation which in the early stage of the disease is extremely hazardous."

This remains true but a greater hazard is probably incurred in the non-recognition of surgical conditions clinically resembling pancreatitis, such as perforation of peptic ulcer, obstruction or strangulation of gut, where immediate laparotomy is desirable. The first step towards safety is the making of a diagnosis.

In 1954, within the short period of a few weeks, there were admitted to this surgical unit five cases of acute pancreatitis, of which only one - the last - was diagnosed before operation. Of the three cases which had been subjected to immediate laparotomy, two died. I thereupon decided to investigate the serum amylase levels present in acute abdominal disease to find out how far the measurement of the blood amylase was of assistance in the diagnosis of pancreatic disease, how far levels comparable to those occurring in acute pancreatitis obtained in non-pancreatic conditions and if the avoidance of laparotomy in the acute stage of the disease constituted a therapeutic advance. The results of investigations in a personally observed series of 23 cases of acute pancreatitis and 200 cases of acute extrapancreatic abdominal disease are presented here.

The non-operative treatment of acute pancreatitis has been directed mainly toward the reduction of pancreatic secretion, on the assumption that the effects of acute pancreatitis are due to the digestive action of pancreatic ferments which have escaped from the ductal system into the tissues and the circulation. Several drugs used for this purpose have appeared to have a beneficial effect upon the course of the illness but, in some cases at least, the evidence that this has been due to an effective suppression of pancreatic secretion is rather inconclusive. Using the serum amylase as a measure of the spill of pancreatic ferments, I have attempted to determine the effect of several therapeutic substances upon series of rats both in normal health and also after the production of obstructive pancreatitis.

PART ONE

SERUM AMYLASE IN HEALTH

1. THE PHYSIOLOGY OF AMYLASE:

   (a) Source and Function of Amylase

   (b) Control of Exocrine Secretion of Pancreatic Amylase

   (c) The Pancreas and Serum Amylase

   (d) The Kidney and Serum Amylase

   (e) Other Factors in Control of Serum Amylase Level

2. BIOCHEMICAL METHODS USED IN THE SERUM AMYLASE ESTIMATIONS:

   (a) Iodometric Method

   (b) Saccharogenic Method

3. SERUM AMYLASE LEVELS IN HEALTHY HUMANS:

   (a) Blood Levels in 100 Controls

   (b) Day to Day Variations

   (c) Diurnal Variations

1. THE PHYSIOLOGY OF AMYLASE

(a) Source and Function of Amylase

Human amylase is synthesised in the pancreas, salivary glands, sero-zymogenic glands of the small intestine and possibly also in the liver (Cohen, 1924). Most of this amylase is secreted directly into the alimentary tract where it aids digestion by splitting starch, glycogen and other carbohydrates as far as the dissacharide stage. The main and most important part of this amylase comes from the pancreatic juice.

Serum amylase is also secreted by these organs direct into the blood stream. Amylase appears in the blood stream shortly after birth and increases gradually in amount so that at the end of the first year it reaches its final adult level (Korowin, 1873). Thereafter, although its level varies from individual to individual, it remains fairly constant in the one person. Wohlgemuth (1909), Elman, Arneson and Graham, (1929) and others have observed that some fluctuation in serum amylase level may occur in health but Somogyi (1931) found individual variations to be not more than 100 per cent. The serum amylase level is unaffected by eating, starvation, drinking, thirst or sleep (Somogyi, 1941) nor in health is it

altered by vagal or hormonal stimulation of the pancreas. It is however reduced after extirpation of the pancreas both in the experimental animal (Schlesinger, 1908) and in man (Cattell and Warren, 1953).

The function of the blood amylase is uncertain. As its level can be altered by induced changes in carbohydrate metabolism (Myers and Reid, 1933; Somogyi, 1941; Dreiling et al., 1958; and others) and by disturbances in liver function (Somogyi, 1934), it may play a part in carbohydrate metabolism. This however has never been proved.

(b) Control of Exocrine Secretion of Pancreatic Amylase

Amylase is a component of the pancreatic juice, which is an abundant, clear, colourless alkaline fluid (pH 8.3 - 8.6), consisting of water, electrolytes and digestive ferments concerned with the breakdown of fat, carbohydrate and protein. Lipase and amylase are secreted in an active form, while the proteolytic enzymes, trypsin and chymotrypsin are mainly secreted as inactive trypsinogen and chymotrypsinogen. These enzymes are produced in parallel concentrations and this quantitative relationship is not altered in health or disease (Baxter, 1935; Agren and Lagerlof, 1936; Harper and Raper, 1943; Doubilet, 1958).

The pancreatic secretion in man is continuous but modified by nervous, hormonal, and vascular influences. The increased pancreatic secretion that appears during digestion occurs in response to specific stimuli, either with the act of eating (cephalic phase) or with the presence of food in the intestinal tract (gastro-intestinal phase). The cephalic phase has, since Pavlov, been attributed to vagal, i.e. neurogenic impulses, while the gastro-intestinal phase was recognised as hormonal following the isolation of secretin by Bayliss and Starling in 1902.

(i) The Neurogenic Control of Pancreatic Secretion: The pancreas has both a sympathetic and a parasympathetic innervation. The sympathetic fibres reach the pancreas through the greater and lesser splanchnic nerves, arising from 5-10 thoracic ganglia, while the parasympathetic nerves reach the gland through the vagi and terminate through intrinsic pancreatic ganglia. All nerves, both sympathetic and parasympathetic, pass through the coeliac plexus, where the two types of fibres cannot be separated anatomically (Blades, 1953). The vagal branches to the pancreas terminate on acinar cells, islets, and smooth muscles of ducts. Pavlov (1897) showed that, in the dog, division and immediate stimulation of the peripheral portion of the vagus caused

inhibition of pancreatic secretions but, if 4-8 days were allowed to elapse after division of the nerve to allow degeneration of the inhibitory fibres, then stimulation of the peripheral portion of the vagus caused secretion of a juice rich in enzymes. This effect can also be obtained by the administration of parasympathomimetic drugs, for example, pilocarpine. The splanchnic nerves also pass through the coeliac plexus and contain both secretory and inhibitory fibres. The inhibitory fibres are adrenergic and probably act by producing tonic changes in the vascular bed of the pancreas (Kuntz and Richins, 1949). The secretory fibres can be paralysed with atropine and Babkin et al (1939) provided direct evidence that they were cholinergic by demonstrating an increased acetylcholine content in blood from the pancreatic vein following splanchnic stimulation. In man, thorace-lumbar sympathectomy has little effect upon pancreatic secretion (Pfeffer, Stephenson and Hinton, 1952) and in practice the splanchnic nerves appear to play an insignificant part in the control of pancreatic secretion (Cattell and Warren, 1953). The presence of a local reflex mechanism was anticipated by Heidenhein in 1875. He postulated that the extrinsic nerves of the pancreas did not act as direct secretory nerves but were merely regulatory in

their function like the nerves of the heart. Kuntz and Richins (1940) have demonstrated the presence of a pancreatic-intestinal reflex which could function after division of splanchnics and vagi but required the integrity of the coeliac plexus. It may be that mechanical and chemical stimulation by food in the duodenum produces pancreatic secretion through such a local reflex (Earl Thomas, 1950).

(ii) The Hormonal Control of Pancreatic Secretion. In 1902, Bayliss and Starling isolated from the mucosa of the duodenum and upper jejunum a substance which stimulated pancreatic and biliary secretion in the denervated organ and to which they gave the name "secretin." This crude secretin has since been fractionated into several components, of which only two have a direct effect upon the pancreas:

1. pure secretin (Mellanby, 1925) which stimulates bicarbonate and water secretion but has no effect upon the enzyme fraction of the pancreatic juice;
2. pancreozymin (Harper and Raper, 1943) which stimulates enzyme production with little effect upon water or bicarbonate production.

These hormones are liberated into the portal vein from the

mucosa of the duodenum and upper jejunum by the stimulus of food or hydorchloric acid entering the duodenum. The physiology of these hormones has been reviewed in detail by Grossman (1956).

(iii) Vascular Factors. The secretory activity of the pancreas demands a blood flow adequate for the metabolic requirements of the glandular epithelium and also for the filtration of sufficient water and electrolytes. It is very doubtful, in the human at least, whether other than gross alterations in blood flow can determine the volume and nature of the external pancreatic secretin (Tankel and Hollander, 1957).

(c) The Pancreas and Serum Amylase

According to Janowitz and Hollander (1951) there is a physiological partition of digestive enzymes, including those of the pancreas, with a large "exocrine" fraction which is secreted into the bowel, and a small "endocrine" fraction which passes direct into the blood stream. There is, under basic conditions, a constant quantitative relationship between these two fractions but this quantitative parallelism may be upset by stress, local disease, and other factors. Measurements of the amylase content of the external pancreatic

secretion and of the urine would indicate that not more than one per cent of the enzyme pancreatic secretion passes direct into the blood stream.

There is lack of agreement as to how much of the amylase normally present in the blood originates from the pancreas. When, however, the pancreatic duct is ligated in animals, there is a rapid rise in the serum amylase to many times its previous level and this excess amylase in the blood is from the pancreas, which now undoubtedly supplies by far the greatest part of the amylase in the blood stream. This increase in the blood amylase is due to a break-down in the physiological endocrine-exocrine partition following the induced obstruction to the ductal secretion so that water, electrolytes, and enzymes flood into the interstitial and peripancreatic tissues in amounts which may be visible to the naked eye as swelling and oedema.

Herring and Simpson (1909) demonstrated by a carmine gelatine injection technique the presence of fine canaliculi between the alveolar cells, which they believed was the enzyme and fluid pathway into the interstitial tissues. The presence of these canaliculi has been confirmed by Rich and Duff (1936) and by Egdahl (1958). Alterations in

permeability of gland parenchyma in acute pancreatic disease may be demonstrated by pancreatography. In the normal gland only the ducts are outlined, whereas in acute pancreatitis the whole gland substance becomes opaque. With recovery from the acute attack the gland returns to its normal semipermeable state with a normal pancreatogram (Doubilet, 1958).

The enzymes pass from the interstitial tissues into the blood both by the pancreatic veins and by the lymphatics (Popper and Necheles, 1940; Aoyama, 1955). Recently Egdahl (1958) demonstrated that initially most of the enzyme is absorbed into the circulation via the pancreatic veins but as the retro- and intra-peritoneal effusion progresses, the amount carried by the lymphatics increases until it surpasses in volume that of the portal blood.

(d) <u>The Kidney and the Serum Amylase</u>

The amount of amylase in the blood is dependent upon the balance between the secretion of amylase into the bloodstream by the pancreas and other amylase-producing organs and the excretion by the kidneys. When there is excessive amylase spill into the blood stream, as in pancreatitis or mumps, there is a sharp rise in the urinary amylase output which

remains increased until the blood amylase has returned to normal levels. While the daily urinary output of amylase in health varies widely with the individual and from day to day in the same person (Smith and Roe, 1952), the amount excreted by the kidneys in one hour is greater than the amount in 100 ml. blood (Gray and Somogyi, 1937).

When there is defective renal function, there is reversal of this ratio with retention of amylase in the blood and serum amylase levels as high as 1,200 Somogyi units (mg. glucose per cent) have resulted from renal causes (Probstein and Pareira, 1952). When such amylase retention occurs, there is usually an accompanying azotaemia (Rodriquez-Olleros, 1955) but Sachar and Weinhaus (1955) have reported one case in which a persistent raised serum amylase level was due to impaired renal clearance for amylase without other evidence of defective renal function. They accordingly advised that in unexpected hyperamylasaemia the urinary amylase should also be determined.

(e) Other Factors in Control of Serum Amylase Level

Even in the absence of disease of the pancreas, salivary glands or of the kidneys, the amylase concentration in the blood may be altered by impairment of liver function, marked

changes in carbohydrate metabolism and by adreno-cortico-thalamic disorders. The evidence for this is as follows:

i. The serum amylase falls in the presence of liver disease (Somogyi, 1934; Gray, Probstein and Heifetz, 1941).

ii. The blood amylase level in subjects without pancreatic disease is altered by the administration of drugs that affect carbohydrate metabolism (Dreiling et al., 1958). In general, the blood amylase falls in states during which there is increased utilisation of sugar and rises following the administration of drugs which diminish the utilisation of sugar. Dreiling and his co-workers have concluded that the normal blood amylase is regulated by extrapancreatic processes.

iii. The response of the blood amylase to upsets in carbohydrate metabolism is dependent upon an intact hypothalamus (Babkin, 1935) and is mediated through the vagus, being abolished by bilateral vagotomy.

An intact pituitary-adrenal axis would also appear to be necessary for normal serum amylase levels. Cope et al. (1939) found that both adrenalectomy and removal of the anterior pituitary in dogs caused significant rises in the serum amylase which could be controlled at least partially

by the giving of extracts of adrenal cortex.

In the rat, hypophysectomy causes atrophy of the pancreas which is partially prevented by the administration of cortisone. (These effects are not specific in the pancreas, for similar changes occur in the peptic cells of the gastric mucosa and in other organs (Baker, 1955)). It would seem likely that the pancreas, like most of the endocrine glands, has a secretory centre in the diencephalon subject to influence by the autonomic nervous system, the hypophysis and the cerebral cortex (Imbriano, 1958). The clinical finding that significant rises in the serum amylase occur in about 20 per cent of patients with cerebral trauma (Smolik, Nash and Ninecurt, 1953) may well be due to disturbance of this centre.

2. BIOCHEMICAL METHODS USED IN SERUM AMYLASE ESTIMATIONS

Various biochemical methods have been evolved for the quantitative estimation of the amylase content of blood. For the most part they consist of incubation of serum with a starch substrate and a measurement of the chemical or physical properties of the degradation products. When starch is hydrolysed by the enzyme amylase there is a random attack upon the polysaccharide chain yielding hexose units of varying lengths and this is accompanied by the following three easily demonstrable phenomena:

i. "dextrinization," with changes in the colour formation with iodine;

ii. "saccharification," i.e. the production of fermentable sugars;

iii. "liquefaction," i.e. decrease in viscosity.

All these phenomena have been used for the measurement of the amylase.

i. Iodometric Method. This method depends upon the fact that starch gives a blue colour which is not shared by its degradation products. This is the basis of the methods developed or modified by Roberts (1881); Wohlgemuth (1908); Somogyi - Iodometric (1938); Comfort and Osterberg (1940); and others.

ii. Saccharogenic Method. This method is dependent upon the accumulation of sugar as shown by the determination of the amount of copper reduction after a given period of hydrolysis. It has been developed by Sherman et al (1910); Myers and Killian (1917); Somogyi - Saccharogenic (1938); and others.

iii. Viscometric Method. Northrop and Hussey (1923) conceived the idea of measuring the viscosity of solutions containing enzymes (trypsin, pepsin) during the period of action of the enzymes and they found that the change in the viscosity of the substance was proportional to the amount of enzyme in the solution. Davidson (1925) modified this technique to determine serum amylase and this was further developed by Elman and McCaughan (1927). These workers claimed that there were theoretical objections to the previous two methods in that with the random attack of the enzyme upon starch, sugar may occur before all the starch has disappeared; conversely the amylase may hydrolyse all the starch to dextrin before any sugar appears. They believed that the viscometric methods did not have these disadvantages.

Dezzi (1940) re-evaluated the methods and found the saccharogenic method to be the most reliable. In the following studies, Somogyi's Iodometric Method has been chosen as the method for the routine investigations on account of its speed and simplicity. Where more accuracy was required, as in the experimental work, a slightly modified Somogyi's Saccharogenic Method has been used.

(a) Somogyi's Iodometric Method (1938).

The time required for complete digestion of a fixed amount of starch (4 ml. starch solution containing 3 mg. starch) when incubated at 37°C. with a certain amount of serum or fluid was determined by periodic testing with iodine. As digestion proceeded the blue colour diminished to be gradually replaced by the red-brown colour of erythrodextrin. The end point was reached when there was no longer any trace of blue and a clear red-brown colour prevailed. The specimen was viewed in transmitted light, the standard source being a 100 watt pearl electric bulb in a microscope lamp holder with the window reduced to a narrow slit.

It was shown by Huggins and Russell (1948) that not over 45 per cent of the substrate should be hydrolysed in the reaction if correct values were to be obtained. On this

account, when the reading was less than four minutes, the serum was diluted to give a value of four minutes or over.

The result was calculated using the formula:

$$\text{Serum amylase (units per 100 ml.)} = \frac{k}{t \times v}$$

where t = time required for digestion in minutes;
v = volume of serum in the mixture;
k = 1600.

"k" is an arbitrary constant which Somogyi used to bring his results by the iodometric method into line with these obtained by the saccharogenic method.

Van Loon, Likins and Seger (1952) described an electric photometric method for estimating the change in the blue starch to iodine colour. This method was tried, as it was hoped that the elimination of error due to the individual variation in colour appreciation would increase the accuracy of the results. It was found rather complicated for routine use, but was found to be of value when the serum was icteric.

(b) Somogyi's Saccharogenic Method (1938)

This was the method of choice in the experimental work. It was performed as described by Somogyi (1938), except for the use of different protein precipitants as suggested by Talluto (1954), viz.:

> At the end of the 30 minute period of incubation of serum and starch-sodium chloride solution, the enzymatic process was arrested by the addition of 1 ml. 2/3 N sulphuric acid and 1 ml. 10 per cent sodium tungstate, instead of 1 ml. 5 per cent copper sulphate and 1 ml. 6 per cent sodium tungstate as originally recommended. Using this modification, the subsequent filtration is more rapid and the filtrate is clearer.

Using this saccharogenic method, Somogyi defined his unit of amylase activity as "the amylase content of 100 ml. serum or fluid which, acting on a starch substrate, releases reaction products which have the reducing power equivalent to 1 mg. glucose."

When estimations were performed in duplicate using these two methods, it quickly became apparent that there was a considerable discrepancy between the results, the values obtained by the iodometric method being about four times higher than those obtained by the saccharogenic method. To distinguish between these different unit values, the former will be referred to throughout this thesis as "iodometric units" and the latter as "Somogyi units" or "mg. glucose per cent."

3. SERUM AMYLASE IN HEALTHY HUMANS

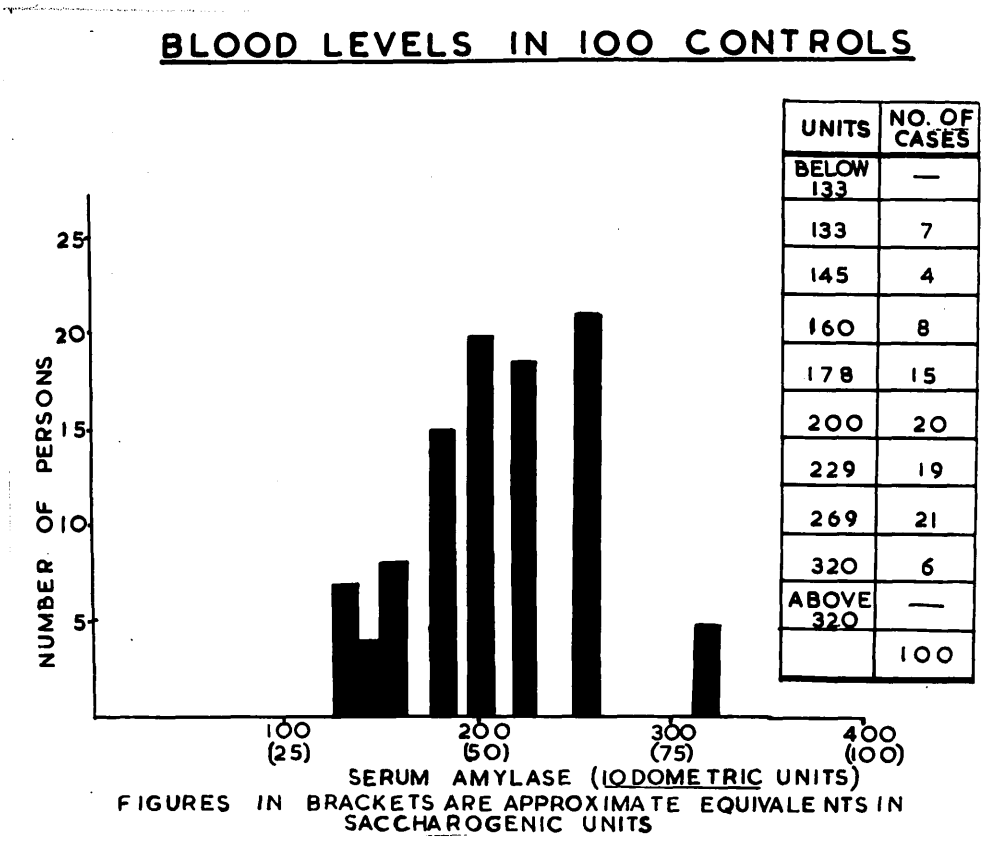
Early in the course of this investigation it was appreciated that the results obtained here by Somogyi's iodometric method (1938) were not comparable with those obtained in his laboratory. I therefore established my own standard of normality using the serum amylase results found in 100 healthy persons. Blood samples were taken from blood donors and from patients without systemic disease or any condition liable to affect the serum amylase level, for example, those with herniae, varicose veins and similar conditions.

A small study was also carried out on variations in the amylase content of the blood occurring during the day or during successive days. In these investigations the saccharogenic method was used, as it was believed to be more accurate. These measurements were carried out on myself and on co-operating medical colleagues, while we were fully engaged on our normal duties. The results found therefore are not necessarily those which would be obtained if these experiments had been carried out under basal physiological conditions.

(a) Blood Levels in 100 Controls (Table I)

The range is 133-320 iodometric units, with a mean of 204 iodometric units and a standard deviation of 50 iodometric units. This range is similar to that obtained by Comfort and Osterberg (1940) and Candel and Wheelock (1946). When these 100 cases were analysed in terms of sex and age, no significant variation in serum amylase was found to occur with either sex or age.

TABLE I



UNITS	NO. OF CASES
BELOW 133	—
133	7
145	4
160	8
178	15
200	20
229	19
269	21
320	6
ABOVE 320	—
	100

(b) Day to Day Variations

Repeat readings of the serum amylase levels on five successive days were made on two subjects. Each day the blood was taken off between 9.00 a.m. and 9.30 a.m. and stored in the refrigerator until tested within the next few hours.

Table II. Comparison of Results on Successive Days

Person	D A Y S					Mean	Range	S.D.
	1	2	3	4	5			
D.W.S.	73	50	42	58	58	56	42-78	10.3
J.R.K.	74	84	74	58	71	72	58-84	9.5

It will be observed that with each subject similar values were obtained on three out of five days. On the remaining two days there were divergences of 12-17 units both above and below the mean value over the five days in each case. It is believed that these divergences are greater than can be accounted for by experimental error and that they represent physiological variations in response to still unrecognised stimuli.

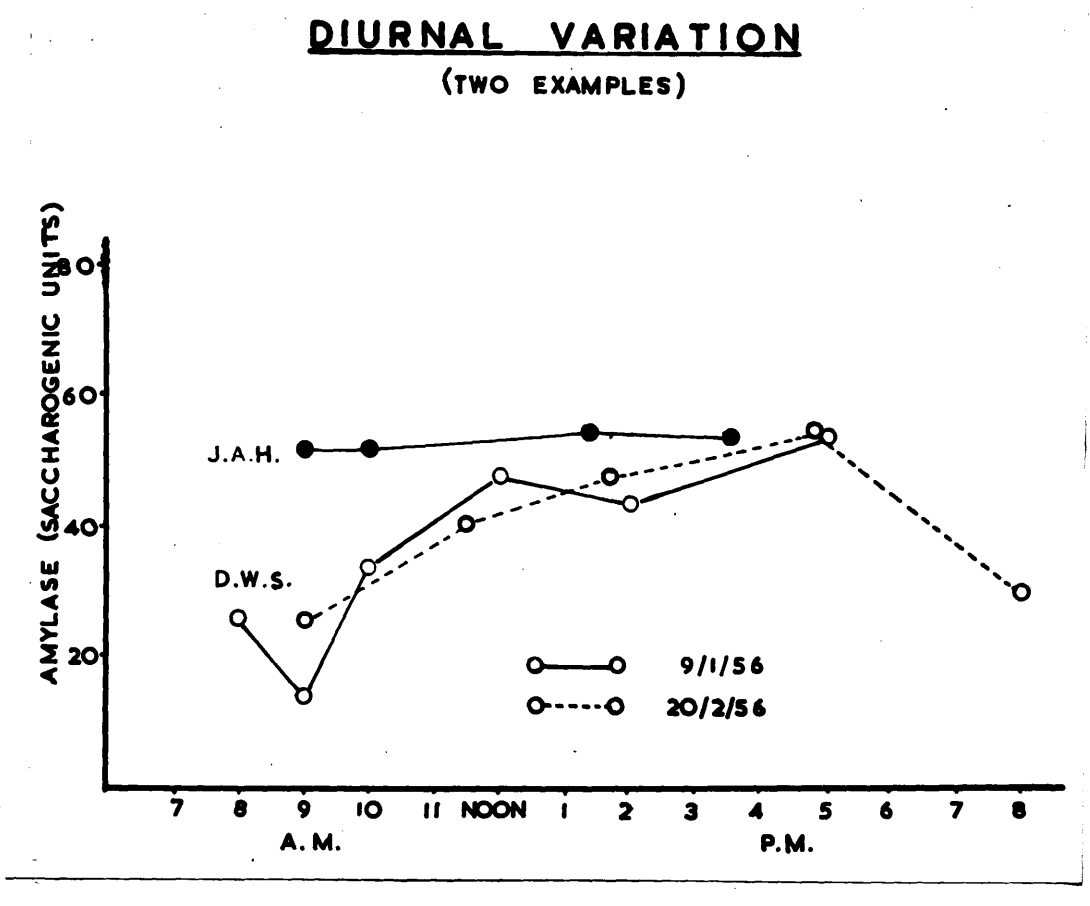
(c) Diurnal Variations

Following the finding of daily inconstancy, the matter was pursued further to find whether the amylase content of the blood significantly varied from hour to hour. Specimens of blood were withdrawn at intervals throughout the day in four healthy subjects and in one of these (D.W.S.) the test was repeated after an interval of six weeks. The results obtained are given in Table III. Two representative examples are shown in the accompanying graph (Fig. 1).

TABLE III. Diurnal Variations in Serum Amylase Level (using Saccharogenic Method)

Subject	A.M.						P.M.											Range	Mean
	8	9	10	11	12	1	2	3	4	5	6	7	8	9					
M. G.		55			42			61			58							42-58	54
W. McQ.		90	96		90		102				74							74-102	90
J. A. H.		52	52					55	55									52-55	54
D.W.S.(1)	26	14	55		48		44				54							14-55	40
D.W.S.(2)		26	41		48						55			29				26-55	40

Fig. 1


DIURNAL VARIATION
(TWO EXAMPLES)
AMYLASE (SACCHAROGENIC UNITS)
80
60
40
20
J.A.H.
D.W.S.
9/1/56
20/2/56
7
8
9
10
11
NOON
1
2
3
4
5
6
7
8
A.M.
P.M.

The first example (J.A.H.) shows a very constant blood level over the eight hours between 8.00 a.m. and 9 p.m. In the second example (D.W.S.) wider variations are apparent and this pattern is closely repeated in the second series of tests.

As the pattern of the diurnal variations shown in this second example resembles that occurring in the eosinophils in the peripheral blood and in the 17-hydroxycorticosteroids present in the blood and urine (Doe, Flink and Flink, 1954), it was suspected that serum amylase levels might be reflecting variations in adreno-cortical activity. Further support for this view appeared to come from the fact that I found abnormally high serum amylase levels in women who had had bilateral adrenalectomy for breast carcinoma and who were on maintenance doses of cortisone. However, the same general pattern in the amylase content of the blood was found in other volunteers but eosinophil counts of the blood taken at the same time showed no close correlation. In other experiments (which are not detailed here) neither adrenocorticotrophic hormone, given as in the Thorn Test (Thorn et al., 1948) nor cortisone 100-150 mg. orally had any consistent or significant effect upon the serum amylase levels. These preliminary attempts on my part to correlate amylase levels in the blood with adrenocortical activity are inconclusive and the subject requires further investigation.

Conclusions

1. In this laboratory the serum amylase measurements in 100 healthy controls ranged from 133 to 320 iodometric units, with an arithmetic mean of 204 iodometric units and a standard deviation of 50 iodometric units.

2. While the level of the amylase in the blood of an individual usually remained fairly constant, divergences of as much as 65 per cent from the mean took place from day to day, or from hour to hour. These fluctuations were more apparent in some persons than others but all deviations were within normal limits for health.

PART TWO

SERUM AMYLASE IN ACUTE PANCREATITIS AND OTHER ACUTE ABDOMINAL DISEASE

1. ACUTE PANCREATITIS

   (a) The Basis of Diagnosis

   (b) The Material Presented

   (c) Serum Amylase Levels Found in Acute Pancreatitis

   (d) The Incidence of Acute Pancreatitis

   (e) Disease of the Biliary Tract and Jaundice in Acute Pancreatitis

   (f) Treatment of Acute Pancreatitis

   (g) The Acute Relapse

   (h) The Mortality Rate, with Special Reference to Non-operative Treatment

2. OTHER ACUTE ABDOMINAL CONDITIONS

   (a) Acute Disease of the Biliary Tract

   (b) Perforated Peptic Ulcer

   (c) Acute Appendicitis

   (d) Other Conditions

3. DISCUSSION

1. ACUTE PANCREATITIS

Fitz in 1889 studied in detail the diagnosis of acute pancreatitis and reached the conclusion that "acute inflammation of the pancreas is both a well-characterised disease and one which is much more frequent than is generally thought .... and it has been repeatedly confounded with acute intestinal obstruction and has thus led, in several instances, to an ineffective laparotomy."

It was however not until several decades later that biochemical methods for the measurement of excessive amounts of pancreatic enzymes in the urine and blood were evolved and attempts at evaluation made. It then appeared that by the development of these methods diagnosis might be achieved with a precision which previously could be reached only by laparotomy. In addition these biochemical methods were instrumental in making possible the wider recognition of a mild form of acute pancreatitis. This acute oedematous pancreatitis or acute interstitial pancreatitis was first recognised as a clinical entity by Zoepffel in 1922. In 1933, Elman comments upon its frequency as occurring three times more often than acute haemorrhagic or necrotic pancreatitis - a much more serious and lethal condition.

The differentiation, on clinical grounds alone, between the two types of disease may be very difficult and indeed impossible. Starr (1955) has suggested that in acute oedematous pancreatitis, where the pathological changes are due mainly to an outpouring of substrate into the gland itself, the clinical features are those of an intra-pancreatic condition, namely pain in the hypochondrium or epigastrium and tenderness over the pancreas, especially the tail. In acute haemorrhagic pancreatitis, on the other hand, there is also extrapancreatic pathology with shock, pain in the iliac fossae and ileus presenting as clinical features.

It has now been recognised that in fact many cases of acute pancreatitis which appear superficially on laparotomy to be of the oedematous type, have macroscopic or microscopic evidence of haemorrhage or necrosis within the gland substance. On pathological as well as on clinical grounds, therefore, the differentiation between the two types is often not clear-cut.

(a) The Basis of Diagnosis.

In arriving at a diagnosis of acute pancreatitis, I have chosen to regard as significant the presence of (i) clinical features characteristic or suggestive of the disease, supported by (ii) significant elevation of the serum amylase and/or (iii) the appearance of the pancreas and peritoneum at laparotomy.

(i) Clinical Features. In its characteristic form acute pancreatitis presents as a sudden abdominal emergency, often alarming in its intensity. The onset is sudden with severe epigastric pain which radiates transversely across the abdomen and into the left flank. There is retching, vomiting, cyanosis, shock, epigastric tenderness, but there is surprisingly little abdominal rigidity. As the disease progresses, increasing abdominal distension is often a feature and a scout film of the abdomen at this stage often shows ileus of a segment of bowel adjacent to the "inflamed" pancreas.

With increased appreciation of the many possible variations in the underlying pathological process, came the recognition that clinical patterns other than the above "characteristic" form do occur. Paxton and Payne (1948) have usefully graded the disease into five clinical types according to severity,

predominant features, and similarity to other diseases. Briefly, the five types are as follows:

Type I. Simulating perforated peptic ulcer, with acute catastrophic onset.

Type II. Simulating cholecystitis (with or without jaundice.

Type III. Simulating intestinal obstruction.

Type IV. Simulating acute alcoholism with gastritis.

Type V. Epigastric mass (pseudocyst) with history of abdominal pain several weeks before.

Pseudocyst formation and suppuration are late signs of acute pancreatitis. Also occurring later, seldom before the third or fourth day of the illness (Cattell and Warren, 1953) and rarely (Cadman, 1958), are signs of retroperitoneal haemorrhage - extravasated blood surfacing at the umbilicus (Cullen's Sign) or appearing in the flanks (Grey Turner's Sign).

(ii) Elevated Serum Amylase. It is apparent from the literature that mere elevation of the serum amylase above the normal range is insufficient evidence of acute pancreatitis. Bockus and Raffensperger (1948) suggested that a serum amylase level five times the upper limit of normal might represent a level above which acute pancreatitis could be diagnosed with reasonable confidence. I have tentatively accepted a serum

amylase level of five times the upper limit of normal (1,600 iodometric units in our laboratory) as a level which strongly supported a diagnosis of acute pancreatitis.

(iii) Appearance at Laparotomy. At laparotomy, diagnosis may be made on (1) the appearance of the pancreas and peripancreatic tissue, (2) the presence of fat necrosis or (3) the nature of the peritoneal exudate.

(1). In the interstitial form of acute pancreatitis, the gland, in part or in whole, shows pallor, swelling and glassy induration. The lobulation may appear more prominent than usual and, if the overlying peritoneum is elevated with fluid, the edges of the gland may be ill-defined. When there has been early massive haemorrhage, the gland may present as a large retroperitoneal haematoma. Usually areas of oedema, necrosis and haemorrhage are scattered irregularly throughout the involved portion. Grey-black areas of necrosis may involve the whole or major part of the gland or appear as spotty areas with intervening normal or oedematous pancreatic tissue. In later stages there may be liquefaction or cyst formation in the necrotic regions.

(2). The presence of fat necrosis within an abdomen is evidence of acute pancreatic disease. It appears as hard whitish-yellow nodules surrounded by inflammatory haloes

and is usually most apparent on the surface or within the pancreas but areas of fat necrosis may also appear in the mesentery, omentum or retroperitoneal fat. Rarely there is fat necrosis of the subpleural and pericardial fat, the lipase apparently travelling along the lymphatic pathways (Perry, 1947). The areas of fat necrosis may disappear slowly or remain permanently as calcified memorials. There is no close relation between the amount of fat necrosis present and the severity of the pancreatic damage.

(3). In haemorrhagic pancreatitis there is a haemorrhagic ("prune-juice") exudate of high amylase content. This is regarded by some as pathognomic of the condition (Keith, Zollinger and McCleery, 1950) and abdominal paracentesis has been advocated as a safer alternative to diagnostic laparotomy (Moretz and Erickson, 1954; Pfeffer, Mixter and Hinton, 1958).

(b) Material Presented.

The material presented consists of 23 cases of acute pancreatitis, admitted between 1954 and 1958. They comprise acute pancreatitis (18 cases), acute relapsing pancreatitis (3 cases) and subsiding pancreatitis (2 cases).

Included under the first heading are patients admitted when in their first major attack, although in many cases there was a history of recurrent attacks of digestive upset and upper abdominal pain extending over several years.

In the three cases included under "acute relapsing pancreatitis," there had been previous attacks of severe pain and in two of the cases pancreatitis had been diagnosed at a previous laparotomy.

The two cases of "subsiding pancreatitis" were admitted after recovery from an acute attack of abdominal pain and pancreatitis was diagnosed only at a subsequent laparotomy.

Brief case histories are included in Appendix B and their salient features are tabulated below (Table IV).

TABLE IV. CASES OF ACUTE PANCREATITIS

A. Acute Pancreatitis

No.	Sex	Age	Provisional Diagnosis	Duration of Illness	Jaundice	Biliary Disease	Amylase	Op.
1	F	66	Cholodocho-lithiasis	10 days	Yes	Non-fun. G.B.	1,600	-
2	F	69	? Cor. Throm. ? Pancreatitis	2 hrs,	No	Cl.	4,000	-
3	F	56	Jaundice	5 days	Yes	Cd.	2,133	Later
4	M	68	Cholecystitis	5 days	Yes	-	1,600	-
5	F	27	Pancreatitis	48 hrs.	Yes	Cd.	1,600	Later
6	F	55	Pancreatitis	6 hrs.	No	-	1,600	-
7	F	58	Cholecystitis	Few hrs.	No	?	2,560	-
8	F	78	Pancreatitis	Few hrs.	No	?	2,133	-
9	F	63	Pancreatitis	3 days	No	Cl.	2,667	-
10	M	54	Jaundice	4 days	Yes	Cc.	3,200	Later
11	F	50	Pancreatitis	3-4 hrs.	No	-	1,600	-
12	F	70	Pancreatitis	12 hrs.	No	Cl.	2,133	-
13	F	59	Empyema G.B.	12 hrs.	No	Cd.	1,600+	Later
14	F	20	Pancreatitis	48 hrs.	No	-	3,200	-
15	M	53	Perforated PU	12 hrs.	No	-	1,600+	Immed.
16	F	63	Cholelithiasis	Few hrs.	No	Cl.	1,067	-
17	F	26	Perforated PU	48 hrs.	No	-	* 914	Immed.
18	M	32	Pancreatitis	Few hrs.	No	Cl.	4,571	Later

* Serum amylase level the next morning.

Cc. = Cholecystitis : Cl. = Cholelithiasis : Cd. = Choledocho-lithiasis

TABLE IV (Contd.).

B. Pancreatitis (in Acute Relapse)

No.	Sex	Age	Provisional Diagnosis	Duration of Illness	Jaundice	Biliary Disease	Amylase	Op.
19	F	69	Relapsing Pancreatitis	?	No	-	8,000	-
20	M	66	Relapsing Pancreatitis	12 hrs.	No	-	1,600	Gastrectomy 1956
21	F	68	Relapsing Pancreatitis	3 days	No	-	200	Cholecystectomy 1954

C. Pancreatitis (Subsiding)

22	F	49	Cholecystitis	?	No	-	400	Yes
23	F	49	Cholecystitis	?	No	Cl.	200	Yes

(c) The Serum Amylase in Acute Pancreatitis.

A. In 16 out of the 18 cases admitted in the initial attack, serum amylase levels ranged from 1,600 to over 4,000 iodometric units (Table IV). On some occasions it was considered sufficient to find a level of "over 1,600 iodometric units" and to record it as such. Most of these patients were admitted to hospital within the first two days of the acute illness and a serum amylase level was accordingly obtained within the first 48 hours of the illness. Several patients who had had acute symptoms for a longer time before admission showed evidence of concomitant acute biliary tract disease. Sometimes the pain had changed in character, becoming more severe, penetrating, and constant. It is believed that at this stage the symptoms of acute pancreatitis became superimposed upon those of biliary tract disease.

The remaining two cases of acute pancreatitis had levels of 1,067 and 914 iodometric units. They were:

Case 16: Mrs. B. B., aged 63 years. Admitted shocked with severe pain in epigastrium and right hypochondrium, nausea and retching for a few hours. Serum amylase on admission was 1,067 units falling after 24 hours to 267 units and reaching 178 units in seven days. The condition settled rapidly. A subsequent cholangiogram showed no evidence of gallstones.

Case 17: Mrs. M. McL., aged 26 years. Admitted at midnight with epigastric pain radiating to back for over 48 hours. Immediate laparotomy showed haemorrhagic pancreatitis with fat necrosis and a normal biliary system. The serum amylase level the next morning was 914 units.

It is possible that the peak elevation was missed in both these cases.

B. Two of the three cases with acute relapse had serum amylase levels over 1,600 units. The third case (Case 21) had a low serum amylase level (200 iodometric units) on admission. At this time no glycosuria was observed but recurrent attacks of pain continued and within two years a moderately severe diabetes mellitus had developed.

It is particularly in chronic relapsing pancreatitis, where there is progressive atrophy and fibrosis of the pancreas and increasing dysfunction both of islets and acini, that serum amylase estimations are often of little help in the diagnosis.

C. The low serum amylase values (400 and 200 units) in the two cases admitted with subsiding pancreatitis illustrate the fact that in pancreatitis the serum amylase level frequently returns to normal before the subsidence of the clinical symptoms (Paxton and Payne, 1948).

In all these cases of acute pancreatitis seen in their initial attack, the serum amylase was considerably elevated above the normal range. In 16 out of 18 cases (i.e. 88 per cent of cases), values of five times the upper limit of normal and over (i.e. 1,600 iodometric units and over) were obtained and in the remaining two cases the elevation was about three times normal (1,067 and 914 iodometric units). With relapses, high transient serum amylase levels seem to be usual until there is marked atrophy of the gland and the clinical picture becomes one of progressive pancreatic insufficiency.

Some cases of acute pancreatitis have been found to have normal or subnormal values at time of admission to hospital. This may be due to the fact that in the uncomplicated case hyperamylasaemia is not maintained and the serum amylase drops to more normal levels within 24–48 hours, the fall being before clinical subsidence of the disease. In acute transient oedema of the pancreas, the hyperamylasaemia may be present for only a few hours. Elman (1937), Bockus and Raffensperger (1948), Heifetz et al. (1941) and Malinowski (1952) have each expressed the view that the serum amylase would always be found raised in acute pancreatitis if the blood were examined early enough.

Values as high as 8,000 units have been reported in acute pancreatitis (Richman, 1956) and there is no apparent correlation between the severity of the disease and the degree of elevation. Nor does a fall in the serum amylase always indicate recovery. Occasionally in the most severe and lethal attacks, the blood enzyme values fall markedly due to destruction of the secreting elements in the pancreas or to the formation of a large pseudocyst into which all the enzymes are drained instead of passing into the bloodstream.

(d) The Incidence of Acute Pancreatitis.

These twenty-three cases of pancreatitis were admitted into this surgical unit over a period of 3½ years. In the first year of investigation, when a special attempt was made to measure the serum amylase level in all acute abdominal emergencies, there were eight cases of acute pancreatitis out of a total of 410 surgical cases admitted with acute abdominal symptoms. Thus pancreatitis accounted for 2.0 per cent of acute abdominal emergencies and 0.2 per cent of all surgical admissions.

When the incidence of acute pancreatitis was compared with that of other acute abdominal conditions it was found, during the year studied, that perforated peptic ulcer and acute appendicitis were respectively 6.4 and 26 times commoner than acute pancreatitis. A comparison between these findings and those in other centres is given below (Table V).

The incidence of acute pancreatitis according to sex and age conforms with the usual pattern found elsewhere. There was a wide age distribution ranging from 26 to 70 years. The sex distribution here was 18 females to 5 males. In the collected series published, the sex ratio varies considerably

and the apparently heavy preponderance of females in the present few cases must be accepted with reservation. A preponderant female incidence has been reported by Paxton and Payne (1948), Morse and Achs (1949), Siler and Wulsin (1950), Fallis (1951), and Kaden and Howard (1956). A high incidence of co-existent biliary tract disease is frequently associated with a female majority.

Cattell and Warren (1953) and others have reported that they have found the disease to be more common in males and this state of affairs is said to exist particularly in hospitals that have an unusually high proportion of alcoholics (Bockus and Raffensperger, 1948). In this series a history of habitual excessive alcohol consumption was obtained on only two occasions - one patient was female and the other male.

Bell (1958) combined data from 14 clinical reports of acute pancreatitis to give a total of 537 men and 768 women, i.e. a comparative incidence of 3 women to 2 men.

TABLE V. Comparative Incidence of Acute Pancreatitis

Author	Pancreatitis	Perforation	Appendicitis	Total Acute Abdominal Emergencies
Elman (1942): St. Louis, U.S.A.	(1)	(2)	(10)	-
Grollman, Baum, Moss and Goodman (1951): St. Louis, U.S.A.	45 (1)	13 (0.25)	222 (5)	-
Burnett and Ness (1955): Aberdeen.	14 (1)	31 (2.2)	149 (10.84)	350
McCollum (1955): Newcastle-on-Tyne	28 (1)	128 (4)	491 (14)	-
Short (1958):	8 (1)	51 (6.4)	210 (26)	410

In brackets is the relative incidence with number of cases of pancreatitis expressed as unity.

(e) Jaundice and Disease of the Biliary Tract in Acute Pancreatitis

Jaundice is present in about 25 per cent of cases of acute pancreatitis (Cattell and Warren, 1953), occurring both in the presence and absence of biliary tract disease. The appearance of jaundice and the frequent presence in acute pancreatitis of concomitant acute disease of the biliary tract may, separately or together, obscure the diagnosis and raise problems in the management of acute pancreatitis. It is appropriate therefore at this stage to look into the incidence of jaundice and of biliary tract disease in this series (Table VI) and, after comparison with the experience of others, to consider their significance.

(i) Jaundice. On five occasions, i.e. 21.7 per cent of cases, jaundice was present on admission. In two cases (Cases 4 and 3) subsequent laparotomy confirmed the presence of a stone or stones in the common bile duct. Case 3 was particularly interesting as neither oral nor intravenous pre-operative cholecystograms showed any evidence of biliary calculi but at laparotomy an operative cholangiogram showed that there was a small stone snugly impacted in the ampullary region (Fig. 2). A later post-operative cholangiogram showed that in this case there was a "common channel" permitting biliary

TABLE VI. Cholelithiasis and Jaundice in Acute Pancreatitis

Cholelithiasis	Jaundice		Total	
	Absent	Present		
Present (confirmed at op.)	3	2	5	10
Present (x-ray only)	4	0	4	
Previous cholecystectomy for cholelithiasis	1	0	1	
Absent (confirmed at op.)	4	1	5	11
Absent (x-ray only)	4	2	6	
Not investigated	2	-	2	
Totals	18	5	23	

Figure 2.

Operative Cholangiogram Revealing Ampullary Calculus.

reflux into the pancreatic ducts. In one case (Case 10) no stones were found in either the gall bladder or the biliary ducts at laparotomy and the obstructive jaundice present was attributed to the inflammatory swelling of the head of the pancreas. In the two remaining cases (Cases 1 and 5) diagnosis of cholelithiasis was made on clinical grounds but radiology failed to confirm the diagnosis and therefore in the absence of laparotomy the cause of jaundice in these cases must remain in doubt.

(ii) Disease of the Biliary Tract. In 9 out of the 23 cases of pancreatitis there was radiological or operative evidence of gallstones. One case of relapsing pancreatitis had had a previous cholecystectomy for cholelithiasis. If this case be included, then 41 per cent of the cases of acute pancreatitis had evidence of longstanding biliary tract disease as shown by the presence of gallstones. If the two cases in which cholecystitis was suspected but which had no investigations be included, then the incidence is 45 per cent. In two cases only were there proved stones in the common bile duct.

(iii) Discussion. The incidence of jaundice here corresponds with the figure of 25 per cent given by Koster and Kasman (1934), Fallis (1939) and Richman (1956). Frank

obstructive jaundice may be due to a stone in the common bile duct with resultant spasm and oedema or to inflammatory oedema of the head of the pancreas. Often the icterus is not marked and the cause is less obvious. In some cases there may be partial obstruction to the common bile duct due to oedema and acute lymphadenitis; in other cases the icterus may be due to excess bile pigments from massive retroperitoneal haemorrhage and necrosis. Infrequently it may be due to hepatitis, a response of the liver to the same noxious agent which produced the pancreatitis (alcohol ?) or to increased concentration of pancreatic enzymes reaching the liver by the portal vein (Richman, 1956). Schiller (1941) has reported evidence of fat necrosis in the liver, ascribing it to reflux of pancreatic juice into the biliary tree. The performance of liver function tests in acute pancreatitis has confirmed the presence of impaired hepatic function in some cases (Cattell and Warren, 1953).

Ivy and Gibbs (1952) in a review of the literature found that the incidence of cholelithiasis in acute pancreatitis ranged in the various series of cases from 39-80 per cent, with an average of 55 per cent. Kaden and Howard (1956) found the incidence to be 41 per cent and Cattell and Warren

(1953) reported it as being between 50 and 75 per cent. The findings in my small series of a minimal incidence of 38 per cent, with a probably higher percentage of cases with cholelithiasis (due to the limitations of the standard radiological investigations), fits in with these findings. The failure to visualise a stone by ordinary cholangiography was apparent in one case (Case 3) and McKenzie (1954) and Pollock (1959) have had similar experiences.

This high incidence of disease of the biliary tract among patients with acute pancreatitis is significant. Molander and Bell (1946) in a large autopsy survey of acute haemorrhagic pancreatitis found that disease of the gall baldder was present in one-third of the male and two-thirds of the female patients, an incidence six times higher than in the normal population. In a more recent study, Bell (1958) found that this association was significant only among persons over the age of 40 years and that below this age gallstones "were not an important factor in the aetiology of pancreatitis."

The association between cholelithiasis and acute interstitial pancreatitis is even closer (Bockus and Raffensperger, 1948); Quick (1932) and Cole (1938) found

cholelithiasis present in all their cases of acute interstitial pancreatitis. Conversely, MacKenzie (1954) found necrotic pancreatitis to be less frequent in the presence of biliary disease than in its absence.

It would seem therefore that the pancreatitis associated with gall bladder disease is frequently but not invariably of the less severe and less lethal type. It will on the whole respond well and rapidly to conservative treatment, though there are indications (considered later) that relapses are likely unless adequate interval biliary tract surgery is carried out.

(f) Treatment of Acute Pancreatitis.

In general, the view taken was that, when an accurate diagnosis could be made and when complications requiring surgical intervention were absent, the treatment in the acute phase should be non-operative, and this was successfully achieved in most cases. The regime adopted was (i) adequate sedation, (ii) gastric suction, (iii) antibiotics and (iv) the control of disturbances of metabolism and electrolyte balance. Other measures used in individual cases were (v) sympathetic nerve block and (vi) the giving of certain ganglion-blocking agents. (vii) The place of surgery in the early and late stages of acute pancreatitis will be discussed in detail later.

(i) Sedation. Patients were sedated with adequate doses of pethidine. Morphine was avoided because of its reputed effect upon the sphincter of Oddi. Several investigators, including Pfeffer et al. (1953), Wapshaw (1953) and Bogoch et al. (1954), have demonstrated that serum amylase values as high as those found in acute pancreatitis may occur when morphine is given to healthy persons, especially when the pancreas at the same time has been stimulated to secrete maximally by the giving of food or cholinergic drug.

(ii) Continual Naso-gastric Suction. On diagnosis, a gastric tube was passed and the stomach emptied and kept empty by constant gastric suction. By this means hormonal secretion of pancreatic juice was prevented. It also relieved the retching and vomiting and was a prophylaxis against ileus and abdominal distension in the severe case.

(iii) Control of Metabolic Disturbances and Electrolyte Imbalance. In most cases fluid and electrolytes were given parenterally. In milder cases it was necessary only to supply the daily fluid and electrolyte requirements while oral feeding was in abeyance but in severer cases a more exacting scrutiny of the fluid and electrolyte state was required to correct and maintain the "melieu interior."

In acute pancreatitis there is an early loss of blood volume by the outpouring of plasma, water, electrolytes into the retroperitoneal tissues and the peritoneal cavity. Keith and Watman (1954) found in acute haemorrhagic pancreatitis an average deficit equivalent to 1,100 ml. plasma and 750 ml. red cell mass. Massive and rapid extravasation may be a significant factor in fatal cases (Elliott, 1957) and adequate and correct replacement is important and may be urgent. In none of these cases was plasma or blood deemed essential in the initial stages of treatment.

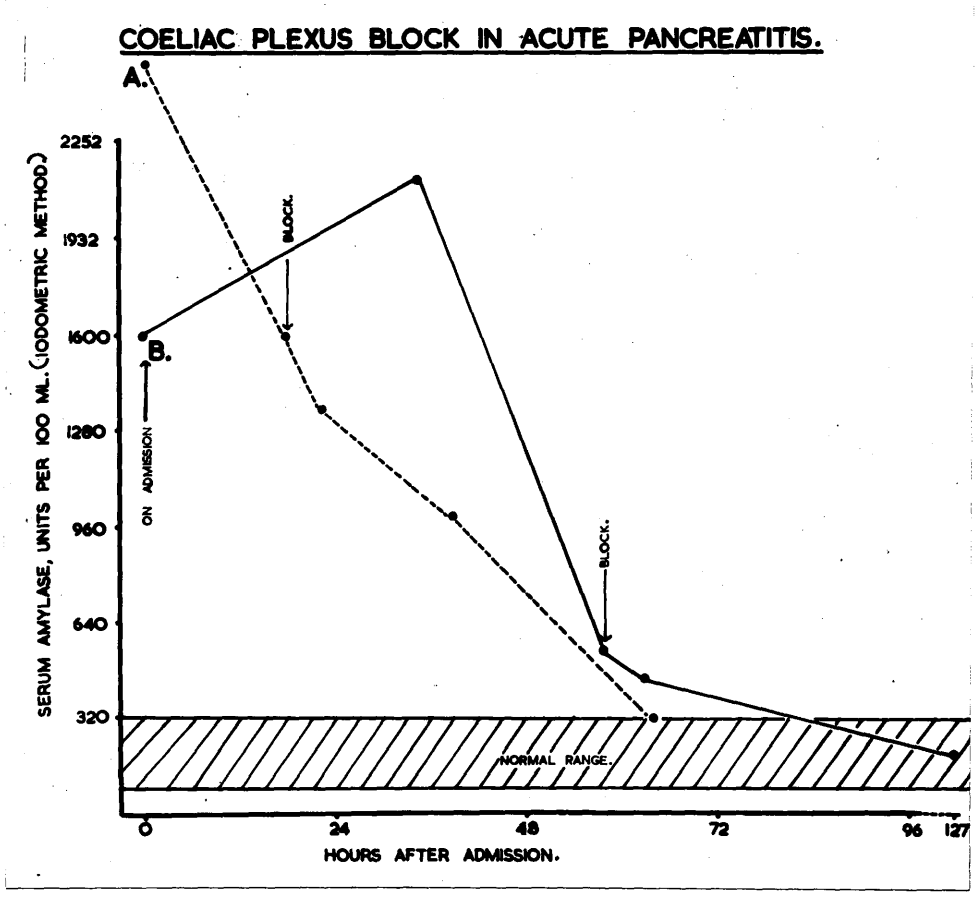
(iv) Antibiotic Therapy. In this series penicillin and streptomycin were given in all but the mildest cases of pancreatitis. There is little evidence for regarding a primary bacterial infection as a frequent cause of pancreatitis but secondary bacterial infection in haemorrhagic pancreatitis is frequent and played a large part in the mortality of pancreatitis before the antibiotics were available (Schweinburg et al., 1953).

Sokolic and Ulin (1957), using artificially-induced acute haemorrhagic pancreatitis in the dog, came to the conclusion that the principal factors causing death in acute haemorrhagic pancreatitis were (1) infection and (2) shock from infection and blood loss. They found that penicillin and streptomycin or aureomycin had a protective value against B. Welchii infection and both lessened the amount of damage to the gland and lowered the mortality rate. Gerber et al. (1958) found in human studies that, while erythromycin, streptomycin and chloramphenicol were excreted in the pancreatic juice, penicillin was uncertain and the tetracycline group was not excreted at all. This differed somewhat from an earlier report by Howard et al. (1951) that penicillin was excreted in high concentration in the pancreatic juice.

(v) Coeliac Plexus Block with Local Anaesthetic. This procedure was performed on three occasions, 15 ml. 1 per cent Xylocaine being injected into either side. In two patients (Cases 6 and 12) abdominal pain had persisted despite twenty-four hours of routine treatment. In both these cases the injection of Xylocaine into the coeliac plexus was followed by permanent alleviation of the pain, though there was no evidence (see Fig. 3) that there had been any specific inhibitory effect upon the enzyme outflow into the blood stream. In the third case (Case 4) no lasting benefit was obtained by this procedure. In all these cases the injection of local anaesthetic was followed by an abrupt fall in the blood pressure enough on two occasions to produce temporary loss of consciousness. I believe that this treatment is potentially dangerous when employed in the acute stage and that it has only a limited use.

Gage and Gillespie (1941) recommended splanchnic block with a bilateral injection of the lower dorsal sympathetic chain with 1 per cent procaine hydrochloride but others have found the effects to be too transient to make the procedure worthwhile. As both the splanchnic and the parasympathetic nerves to the pancreas pass through the coeliac plexus, it would seem not only less unpleasant to the patient but also more effective to infiltrate the coeliac plexus and interrupt the local reflex mechanism, if indeed such a mechanism exists.

Figure 3.


COELIAC PLEXUS BLOCK IN ACUTE PANCREATITIS.
A.
B.
BLOCK.
BLOCK.
ON ADMISSION
2252
1932
1600
1280
960
640
320
SERUM AMYLASE, UNITS PER 100 ML. (IODOMETRIC METHOD.)
NORMAL RANGE.
0
24
48
72
96
127
HOURS AFTER ADMISSION.

(vi) Treatment with Ganglion-blocking Agents. I have used Banthine and Buscopan, but only in a single case each, in attempts to reduce pancreatic secretion and to relieve spasm of the sphincter of Oddi.

Banthine (methantheline bromide) was used in a patient with severe haemorrhagic pancreatitis (Case 4) in doses of 50-75 mg. eight-hourly intravenously, followed by hexamethonium bromide (stocks of Banthine were exhausted) for a total of seven days. No obvious improvement was observed with these drugs and the patient had a long and stormy convalescence with ileus as a prominent feature in the early weeks. Bowel sounds were absent until the seventh day and normal bowel activity was not established until the twelfth day. It was suspected here that the ileus had been increased by the use of a ganglion-blocking agent.

Buscopan (hyoscine N-butyl bromide) was used in a patient (Case 15) who had been re-admitted to a medical ward with an acute relapse. After eight days of pain necessitating frequent sedation, he was given Buscopan 20 mg. four-hourly. Thereafter there was rapid relief of symptoms and the patient was dismissed on the nineteenth day well. As he had had several attacks since his initial attack of acute pancreatitis four months

previously, he was told to continue with Buscopan on dismissal. Two subsequent attacks were attributed by his practitioner to his failure to continue the drug. When last seen he had remained free from symptoms for six months and was taking Buscopan regularly. It is possible however that alcohol may have been an aetiological factor here in determining both the initial acute attack and the subsequent relapses.

(vii) Operative Treatment. Laparotomies were performed on eight occasions and these will be considered according to the phase of the illness, viz:

(a) Immediate on admission and during acute stage of disease;

(b) Delayed until after the subsidence of acute symptoms but on first admission. These cases were in fact operated upon at about 3-4 weeks after admission;

(c) On relapse and at a subsequent re-admission.

It will be seen from Table VII that the indications for operation were (i) diagnostic error or doubt and (ii) the actual or suspected presence of complications.

In the two cases operated upon in the acute stage of the illness, perforation of a peptic ulcer could not be excluded on clinical grounds and a serum amylase reading was not available

TABLE VII. Operations in Cases of Acute Pancreatitis

Stage	Case No.	Indication	Operation
(a) Immediate	17	Diagnosis: ? perforated D.U.	Laparotomy
	15	Diagnosis: ? perforated D.U.	Laparotomy (Appendicectomy)
(b) Delayed	5	Complication: ? subphrenic abscess	Laparotomy, Cholecystectomy
	3	Complication: Jaundice (stone)	Choledochostomy, Cholecystectomy
(c) Relapse	10	Complication: Jaundice (pancreatitis)	Laparotomy, Diln. of sphinc.
	13	Complication: Acute cholecystitis	Cholecystectomy
	22	Diagnosis: ? cholecystitis	Laparotomy
	23	Diagnosis: ? cholelithiasis	Laparotomy, Cholecystectomy

at this stage. On one occasion an incidental appendicectomy, but no other abdominal procedure, was carried out. In the two other cases in which there was diagnostic difficulty, the patients were seen several days after the onset of the illness and serum amylase readings of 200 and 400 iodometric units were not helpful in making a diagnosis of acute pancreatitis.

The other cases were operated upon for suspected peri-pancreatic or subphrenic abscess (Case 5), persistent obstructive jaundice due to a small ampullary stone (Case 3), recurrence of obstructive jaundice due to compression of common bile duct by the swollen head of the pancreas (Case 10) and recurrent attacks of acute cholecystitis (Case 13).

Secondary bacterial infection is not uncommon in acute necrotic pancreatitis (as Case 5 was) and small abscesses in the substance of the pancreas are frequently seen in fatal cases. A large retroperitoneal abscess is much more rare. The onset may be insidious and the abscess may track into the perirenal area or subdiaphragmatic space or may extend into the mediastinum. Clinical evidence of a spreading peritonitis is an indication for laparotomy. Zaslow (1953) reported two cases of perforation of the common bile duct as a complication of acute pancreatitis.

Obstructive jaundice in acute pancreatitis is sometimes stated to be an indication for immediate laparotomy and decompression of the biliary tract on the grounds that, if a common biliary-pancreatic channel were present, distal obstruction would result in the regurgitation of bile into the pancreatic tree with the danger of precipitating or exacerbating pancreatic necrosis. In practice most cases of pancreatitis with obstructive jaundice respond rapidly to conservative treatment with an early fall in both the serum amylase and bilirubin. Moreover, during the acute attack, when the patient has profound systemic upsets and when the structures within the duodenal loop are oedematous, friable and haemorrhagic, exploration of the common bile duct may be difficult, dangerous, and disappointing and the resultant decompression whether by cholecystostomy or choledochostomy rather ineffectual as far as the pancreatic duct system is concerned. If, on the other hand, the acute stage can be tided over, definitive surgical treatment of the disease of the biliary tract is easier and safer.

In obstructive jaundice due to compression of the common bile duct by the acutely swollen head of the pancreas (as in Case 10), surgical exploration is necessary for diagnosis and

treatment, if spontaneous recovery does not take place. Hepatogenous jaundice constitutes a contra-indication to surgical intervention.

Other complications which were not encountered in this series but which are indications for surgery are pseudocyst and intestinal obstruction. In the early stages, obstructive symptoms may be prominent and persistent due to localised ileus of adjacent bowel, usually jejenum but sometimes duodenum, ileum or transverse colon (Cattell and Warren, 1953). More rarely and at a later stage there may be a mechanical obstruction involving the small bowel (de Vidas, 1947; Moore, 1956; Hardy and Bowlin, 1957; Pollock, 1959) or colon (Miln and Barclay, 1952).

(g) The Acute Relapse

Among the twenty-three cases of acute pancreatitis, three were in an acute relapse when first seen and five cases which had been diagnosed and treated when in the initial attack had subsequent relapses. It is not believed that even this rather high relapse rate represents the total, as the follow-up has been short and incomplete.

A. Cases first seen in relapse:

i. Female, aged 69 (Case 19): Has had repeated attacks of pancreatitis over two years. X-ray shows non-functioning gallbladder. Laparotomy refused.

ii. Male, aged 66 (Case 20): Laparotomy in 1953 showed pancreatitis without biliary tract disease. He has had recurrent attacks of pain since, not relieved by cholecystectomy in 1955 (? normal gallbladder).

iii. Female, aged 68 (Case 21): Laparotomy in 1950 showed cholelithiasis and subsiding pancreatitis. Recurrent attacks after cholecystectomy and has since developed diabetes.

B. Cases of acute pancreatitis that have relapsed:

i. Male, aged 54 (Case 10): Uneventful recovery from initial attack of pancreatitis and obstructive jaundice but relapsed weeks later and a laparotomy confirmed pancreatitis and the presence of a non-calculous cholecystitis. Sphincter of Oddi dilated and no recurrence since.

ii. Female, aged 59 (Case 13): had attacks of pancreatitis until cholecystectomy for cholelithiasis in 1957 and has had no attacks since.

iii. Male, aged 53 (Case 15): "Alcoholic" pancreatitis. No evidence of biliary tract disease at laparotomy. Has had four major relapses since first attack in January, 1958.

iv. Female, aged 26 (Case 17): "Post-partum" pancreatitis (Langmade and Edmison, 1951; Joske, 1955a). No evidence of biliary tract disease at laparotomy; not addicted to alcohol. Had a severe relapse 16 months after first attack.

v. Female, aged 49 (Case 22): Laparotomy following a relapse showed a subsiding pancreatitis and no evidence of biliary tract disease. Drinking habits not known.

Kaden and Howard (1956) found a relapse rate of 45 per cent in patients with untreated acute pancreatitis. In their experience relapsing cases could be divided into two groups, distinct in cause, progress and response to surgery: (a) those associated with chronic alcoholism ("alcoholic pancreatitis") and (b) those associated with cholelithiasis ("biliary pancreatitis"). They found that the relapse rate was about the same in the two groups but Bockus, Kalsar, Bagoch and Stein (1955) found that in their series the pancreatitis associated with alcoholism was more severe and more prone to relapse and complications than in those cases in which biliary tract disease and not alcoholism was a factor. Howard et al. (1958) in a large survey of pancreatitis which comprised 350 cases of their own and more than 5,000 cases collected from the literature

found that "alcoholic" pancreatitis eventuates in pancreatolithiasis (intraduct or parenchymatous) in almost half the patients and steatorrhoea and diabetes develops in about a quarter of the cases. Also within this group, cholecystectomy in the absence of gallbladder disease did not prevent recurrence of the pancreatitis. In contrast to this, biliary pancreatitis rarely resulted in pancreatic calcification or diabetes and definitive gallstone surgery characteristically interrupted the course of the disease.

Such a simple cure for relapsing pancreatitis associated with biliary tract disease has not been achieved by all. Joske (1955b), for example, does not accept the concept of "biliary pancreatitis" and found among his 90 cases of acute pancreatitis 14 which had not benefited by surgery of the biliary tract. There is some evidence (Niedner, 1957) to support the theory that the primary lesion may be in the region of the papilla of Vater and that biliary tract disease and pancreatic disease are due to stagnation in the respective duct systems. Doubilet and Mulholland (1956) have reported upon the treatment of 319 cases of recurring pancreatitis by cholecystectomy and sphincterotomy with a recurrent morbidity rate of only 10 per cent in patients followed over two years.

The few cases given here show many of the features generally found in the recurrent attacks of pancreatitis. Among the ten cases of acute pancreatitis which also had gallstones, only one was observed to relapse and she undoubtedly benefited from cholecystectomy. The two cases of acute pancreatitis with associated cholelithiasis which were seen for the first time after they had had several acute attacks remained in statu quo. One declined operative treatment and the other continued to have attacks after cholecystectomy.

Alcoholism was rare among my cases in contrast with its frequency in some large series reported from the United States of America. Howard et al. (1958) in their nationwide survey found that between one-third and one half of all cases of acute pancreatitis could be attributed to alcoholism. Pollock (1959) in a survey of 100 cases of acute pancreatitis in Leeds found no case in which there was an association with alcoholic excess.

(h) The Mortality Rate with Special Reference to Non-operative Treatment.

The mortality rate in this series is nil. In the four cases operated upon during the initial illness, two (both in young women) had generalised haemorrhagic pancreatitis and two showed only acute oedematous pancreatitis with fat necrosis. In the remaining cases acute interstitial pancreatitis was suspected on clinical grounds but no precise information as to the pathological state of the pancreas is available. Accordingly little can be said about the encouraging results, apart from noting that there is nothing here to contradict the view that conservative treatment is best in the acute stage of the uncomplicated case.

The death rate in acute pancreatitis has shown a marked fall in the last few decades. In 1927, when diagnosis was dependent upon laparotomy, Schmieden and Sebening collected 1,278 cases of acute pancreatitis with an overal mortality rate of 51.2 per cent. The present mortality rate probably varies from about 16 per cent (Siler and Wulsin, 1951; Raker and Bartlett, 1953; Fogerson and Shedd, 1955; Thompson and Derrick, 1957) to about 5 per cent (Probstein and Pareira, 1954; Zollinger, Keith and Ellison, 1954).

Both diagnosis and treatment play significant parts in this achievement. As biochemical tests for the detection of excess of enzymes in blood or urine have become available for clinical use, many cases of acute pancreatitis are now recognised which previously would have been missed. This and the consequent increase in awareness of pancreatitis as a not infrequent cause of acute abdominal pain has resulted in the diagnosis of a greater proportion of relatively mild and transient cases of acute oedematous pancreatitis.

Acute oedematous pancreatitis now constitutes 75-80 per cent of most recent series of cases (Siler and Wulsin, 1950; Fogerson and Shedd, 1955; Demetriades and Polayes, 1958). Acute pancreatic necrosis is still a serious disease with a mortality rate which may be as high as 62.5 per cent (Demetriades and Polayes, 1958), whereas the mortality in acute oedematous pancreatitis is almost nil.

The improvement in the recovery rate is also due to improvement in treatment, of which the avoidance of operation in the acute stage is only a part. The prompt restoration of circulating blood volume, control of electrolytes and fluid balance and prophylactic use of antibiotics are therapeutic

measures which strike at the main causes of death in these cases. Since the report in 1952 by Stephenson, Pfeffer and Saypol of the successful treatment of acute haemorrhagic pancreatitis with cortisone in a patient who was gravely ill and severely shocked, numerous other successes with either cortisone or adrenocorticatrophic hormone have been reported (see later). These drugs are undoubtedly lifesaving in the presence of adrenocortical insufficiency and there is also some experimental evidence that they may have a beneficial anti-inflammatory effect in severe haemorrhagic pancreatitis (Stewart, Elliott and Zollinger, 1958).

It is difficult to determine the extent to which selection and improved therapy might have contributed to the lowered mortality rates. Cole (1938) and Richman (1957) have considered the evidence and are satisfied that the avoidance of surgery in the acute stage has led to improved results. This avoidance of surgery, however, is not always possible nor is it invariably desirable. Complications requiring surgery may arise at any time and an error of diagnosis may lead to serious consequences. The place of the serum amylase tests in the differential diagnosis between acute pancreatitis and other acute extra-pancreatic abdominal conditions will be considered in the next section.

2. OTHER ACUTE ABDOMINAL CONDITIONS

In order to assess further the value of serum amylase estimations in the diagnosis of acute pancreatitis, the serum amylase was measured in 200 patients admitted as surgical emergencies on account of pain believed not to be pancreatic in origin. There were 22 cases with amylase levels above the normal range of 133-320 iodometric units, but in no case did the serum amylase level exceed 914 iodometric units.

Hyperamylasaemia was commonest (28.7 per cent) in acute disease of the biliary tract, the overall incidence being 7.3 per cent among the other cases. This would support the belief that disease of the biliary tract is linked more closely with pancreatitis than are other acute abdominal conditions.

The various conditions shown in Table VIII will be considered separately.

TABLE VIII.

Serum Amylase in Extra-pancreatic Abdominal Conditions

Amylase Units (Iodometric)		Biliary Tract Disease	Perforated Peptic Ulcer	Acute Appendicitis	Other Conditions
	914	1	-	-	-
	800	-	-	-	-
	640	-	-	-	-
	533	2	2	1	1
	500	1	1	-	-
	457	1	-	-	-
	400	5	2	4	1
Normal Range	320	2	4	10	5
	269	3	9	6	10
	229	2	5	11	6
	200	-	3	8	6
	178	3	3	9	4
	160	1	2	9	8
	145	2	-	5	3
	133	-	3	3	4
Below 133		12	3	6	8
Total		35	37	72	56
No. with raised amylase (%)		10 (28.7%)	5 (13.6%)	5 (6.9%)	2 (3.6%)
			12 (7.3%)		

(a) Acute Disease of the Biliary Tract.

Thirty-five patients with acute disease of the biliary tract who had had serum amylase measurements made on or shortly after admission comprised:

2 cases of acute non-calculous cholecystitis

12 cases of acute obstructive cholecystitis (biliary calculi present)

6 cases of acute cholecystitis (calculi presumed but unproven)

15 cases of choledocholithiasis.

Results: Table IXA.

The incidence of hyperamylasaemia was higher where calculi were proved to be present. It occurred in 6 out of the 15 patients (40 per cent) with radiological and/or operative evidence of choledocholithiasis.

In none of these cases which had a laparotomy was there any evidence of past or present pancreatitis. Any case of known or suspected disease of the biliary tract, which showed clinical or biochemical features of pancreatitis, was excluded from this group and considered under "Acute Pancreatitis." Among the eighteen cases of acute pancreatitis, there were eight

TABLE IXA. Incidence of Elevated Serum Amylase in Acute Disease of the Biliary Tract

(excluding cases with associated acute pancreatitis)

Condition	No. of Cases	Raised Serum Amylase
Non-calculous cholecystitis	2	-
Cholelithiasis (stones demonstrated)	12	3
Cholelithiasis (unproven [x])	(6)	(-)
Choledocholithiasis	15	6

[x] These were elderly persons who made a rapid recovery from their acute condition and, as operation was not contemplated, investigations were incomplete.

TABLE IXB. Serum Amylase in 45 Cases with Biliary Tract Disease

(including 10 cases of acute pancreatitis)

Condition	No. of Cases	Raised Serum Amylase		
		<1,000 Units	>1,000 Units	Total
Non-calculous cholecystitis	3	-	1	1
Cholelithiasis (stone demonstrated)	17	3	5	8
Cholelithiasis (unproven)	7	-	1	1
Choledocholithiasis	18	6	3	9
T o t a l s	45	9	10	19

with proven biliary calculi, one with an unconfirmed diagnosis of cholelithiasis and one who had obstructive jaundice and a non-calculous cholecystitis, the biliary obstruction being found at laparotomy to be due to pancreatitis. When these cases are included with the above 35 cases, the results are as in Table IXB.

Thus nearly half (42 per cent) of these cases with acute disease of the biliary tract had an elevation of serum amylase and about half of those with raised serum amylase levels showed evidence of pancreatitis.

Consideration of the Literature. The reported incidence of raised serum amylase in acute cholecystitis varies from 33 per cent (McCorkle and Goldman, 1942) and 9 per cent (Hall, Howard, Jordan and Witt, 1956). Both groups of investigators found that in about half of the cases with hyperamylasaemia, no macroscopic evidence of pancreatitis was present at operation.

A symptomless functional upset is sometimes found after morphine injections, or when the pancreatic intraduct pressure is raised during cholangiography (Howell and Bergh, 1950). Serum amylase values as high as 4,000 Somogyi's Units have occurred without the patient experiencing abdominal pain, nausea,

or vomiting (Pfeffer et al., 1953). In all these circumstances a high concentration of enzymes is released into the interstitial tissues creating a situation favourable for the development of pancreatitis, but it is a "prepancreatitis" rather than a pancreatitis.

When the obstruction is more prolonged and more complete, the clinical and pathological picture is of acute interstitial pancreatitis. There is experimental support (Thistlethwaite and Hill, 1952) for the view that obstruction alone produces a rise in the serum amylase, a varying amount of oedema of the gland and sometimes intraperitoneal fat necrosis. This is the type of pancreatitis most frequently found in association with biliary tract disease (Mackenzie, 1954).

The role of the biliary tract in the pathogenesis of acute haemorrhagic pancreatitis is more obscure. In 1850, Bernard produced a condition resembling pancreatitis by the injection of bile and oil into the pancreas of experimental animals. Korte (1898), Lancereaux (1899), and Oser (1903), as quoted by Richman (1957), each independently came to the conclusion that pancreatitis was due to gallstones in the terminal part of the common bile duct acting as a barrier to the outflow of pancreatic juice and allowing bacteria from the inflamed common

duct to enter the pancreatic duct. Opie in 1901 found in a fatal case of haemorrhagic pancreatitis a small gallstone at the ampulla, obstructing biliary and pancreatic outflow yet allowing biliary reflux into the pancreatic ducts. However, in the series collected from the literature by Wapshaw (1955), the incidence of ampullary stone in acute pancreatitis varied from 13.0 per cent to 1.4 per cent with an overall average of 4.9 per cent. Following Opie's report in 1901, there has been extensive and intensive research into the incidence and significance of the "common channel" and of the "bile factor" in acute pancreatitis. The work has been reviewed by Wangensteen, Leven and Manson (1931), Siler and Wulsin (1950), Cattell and Warren (1953), Wapshaw (1955) and Richman (1957). There is also ample evidence that neither a "common channel" nor biliary reflux are necessary for the production of acute haemorrhagic pancreatitis. I have studied the effects of pancreatic duct obstruction, with and without biliary regurgitation into the pancreatic ducts, in the rat and my findings with a further brief discussion are given in Part III,

(b) Perforated Peptic Ulcer

Thirty-seven gastroduodenal perforations were studied and in five of these (13 per cent) the pre-operative serum amylase was raised to between 320 and 533 iodometric units.

In a search for some correlation between the amylase content of the peritoneal fluid and of the blood, a sample of peritoneal fluid was taken at laparotomy in seventeen out of the thirty-seven cases. The results are recorded in Table X along with the site, size and duration of the perforation and the pre-operative serum amylase level. I have established no obvious correlation between the level of the serum amylase and the size or site of ulcer, duration of illness or degree of peritoneal contamination.

Consideration of the Literature. The reported incidence of hyperamylasaemia in gastroduodenal perforations varies from 15 to 50 per cent with an average of about 20 per cent (Table XI).

Pemberton et al. (1950), after experimental studies in dogs, came to the conclusion that post-perforation elevations in serum amylase were due to absorption of amylase from the peritoneum. Musgrove (1950), in eight patients, found a direct relationship between the size of the perforation and the serum amylase. Mahaffey et al. (1955) have confirmed this in their larger series

TABLE X

Amylase Levels in Peritoneal Exudate and in Blood in Seventeen Cases of Perforated Peptic Ulcer.

Case No.	Age (years)	Perforated peptic ulcer			Peritoneal fluid		Blood amylase
		Site	Diameter (inches)	Duration (hours)	Volume*	Amylase units/100 ml.	Amylase units/100 ml.
1	46	Duodenal	¼	2	+	133	107
2	35	Duodenal	⅓	2–3	+	533	267
3	26	Gastric	Not stated	3	++	64,000	400
4	53	Duodenal	,, ,,	4	+	17,204	177
5	31	Duodenal	,, ,,	5	+++	3,200	229
6	53	Gastric	,, ,,	6	+++	400	267
7	76	Duodenal	,, ,,	6	?	26,667	267
8	37	Duodenal	¼	6	++	1,280	114
9	43	Duodenal	⅓	6	+++	20,000	200
10	56	Duodenal	¾	10	+++	23,659	320
11	34	G-E stoma	Small	10	+	25.600	229
12	31	Gastric	Small	12	++	2,822	160
13	27	Duodenal	⅓	12	+++	18,605	267
14	68	Duodenal	Large	14	+++	1,067	94
15	47	Duodenal	Small	15	?	1,943	300
16	68	Duodenal	⅓	?18	++	1,280	229
17	55	Gastric	⅓	36	+++	4,267	533

*Arbitrary scale of measurement ranging from + =small local collection of fluid to +++ =general flooding of peritoneal cavity.

TABLE XI

Incidence of Elevated Serum Amylase in Gastroduodenal Perforations.

Author	Total No. of cases	Raised serum amylase	
		Under 1,000 units No. of cases (%)	Over 1,000 units No. of cases
Probstein *et al.* (1939)	17	4 (24)	–
Wapshaw (1949)	32	7 (22)	–
Musgrove (1950)	20	3 (15)	–
McCollum (1955)	10	5 (50)	–
Burnett and Ness (1955)	31	12 (39)	2
Mahaffey *et al.* (1955)	51	8 (16)	–
Present series	37	5 (13)	–
TOTALS	198	44 (22)	2

of thirty-nine patients and also found that the most significant rises were in perforations occuring within three hours of a meal or the taking of alcohol and when there was a delay of over 12 hours between perforation and test. Wapshaw (1951) in his series of thirty-two cases found no relationship between the enzyme levels in the blood and in the peritoneal fluid and observed that raised blood amylase values were obtained only in cases of peptic ulcer perforated for seven hours or more. Coffey (1958) found no correlation between the duration of the perforation and the level of the serum amylase. It would seem over-simplification to regard the process as one of simple mechanical filtration, for other factors may be important, including alteration in the peritoneal permeability, as the peritoneum responds to the chemical and bacterial irritation from the spilled gastric or duodenal contents.

(c) Acute Appendicitis.

Results: Pre-operative serum amylase levels were measured in seventy-two cases of acute appendicitis and were found to be elevated in five (6.9 per cent). There was no correlation between the amylase level and the duration of symptoms or degree of inflammation in the appendix itself. None of the thirty-three cases without peritonitis, but five out of the thirty-nine cases with peritonitis (12.8 per cent) had serum amylase levels above normal (Table XII). The incidence of raised serum amylase was the same among those with generalised peritonitis as among those with peritonitis limited to the appendicular region, though in the former group the elevations tended to be slightly higher. In none of these cases were very high serum amylase values found.

Consideration of the Literature. Normal serum amylase values in acute appendicitis were found by McCollum (1955) - 14 cases - and by Kovacs et al. (1955) - 50 cases. Burnett and Ness (1955) found elevations in fifty-seven out of 149 cases of acute appendicitis, the highest value being 800 units (Somogyi).

TABLE XII. Appendicitis and the Serum Amylase

Type of Case	No. of Cases	Mean Amylase Level (Iodometric Units)	Raised Serum Amylase	
			No.(%)	Iodometric Units
No Peritonitis	33	193	-	-
Local Peritonitis	23	194	3(13)	400 400 400
General Peritonitis	16	259	2(12.5)	457 533

(d) Other Conditions.

Results: In this final group were collected all other cases with acute abdominal pain, including intestinal obstruction, acute peptic ulcer, infective hepatitis, diverticulitis, non-specific mesenteric adenitis and ectopic pregnancy. In two cases out of the forty-eight examined (4.2 per cent) the serum amylase was over 320 iodometric units - a case of strangulated inguinal hernia (356 iodometric units) and one of infective hepatitis (533 iodometric units).

Consideration of the Literature

(i) Intestinal obstruction has been associated with a raised serum amylase on several occasions. Raffensperger (1951) reported the case of a woman in whom a serum amylase of 343 mg. per cent (2½ times the upper limit of normal) delayed for several days the operative relief of an internal strangulation of bowel. Pollock (1959) reported a case of a man of 36 with intestinal obstruction due to a band, who had a serum amylase level of 1,600 units, and also the case of a woman of 66 with a gangrenous loop of small bowel within a femoral hernia sac, who had a serum amylase level of 2,000 units, although at autopsy the pancreas appeared normal. With the exception of these two cases along with the two cases reported by Burnett and Ness (1955) who had levels of 2,000 and 1,000 Somogyi units,

elevations when present in cases of intestinal obstruction have been moderate and less than 500 mg. per cent (Polowe, 1946; Raffensperger, 1951; Kovacs et al., 1955).

The cause of the hyperamylasaemia is not certain. Ivy and Goldman (1939) have produced spasm of the sphincter of Oddi by distension of the colon. In obstruction with strangulation, the amylase content of the peritoneal transudate is raised considerably. Moretz and Erickson (1954) found the mean elevation to be 11.3 times that of normal blood and it would seem reasonable to suggest that in these cases there is a transperitoneal absorption of amylase as is believed to occur in gastroduodenal perforations. A co-existing peritonitis may also play its part.

(ii) Penetrating Peptic Ulcer. Involvement of the pancreas by a peptic ulcer may cause a moderate elevation in the serum amylase (Comfort and Osterberg, 1940; Heifetz et al., 1941; Lewison, 1941; Malinowski, 1952). Among Malinowski's nine cases of penetrating peptic ulcer, elevations up to 976 Somogyi units (four times the upper limit of normal were found. At one time it was hoped that serum amylase determinations might assist in the recognition of this complication but the results have proved too uncertain to be of value.

(iii) Acute Peritonitis of almost any aetiology may give elevated serum pancreatic enzyme values, the elevation appearing between the fifth and tenth days (Raffensperger, 1951). In the cases of appendicitis reported here, elevations were found only when there was an obvious peritonitis present. In all the cases the modest elevations present were recorded before the fifth day of illness.

(iv) Infective Hepatitis. The finding of a raised serum amylase in a case of infective hepatitis (proved by liver biopsy) is of interest in that it is stated that in liver disease, in contrast to biliary tract conditions, the serum amylase is normal or subnormal and the degree of fall is a measure of hepatic impairment (Somogyi, 1941; Gray et al., 1941).

(v) Ruptured Ectopic Pregnancy. An isolated case of ruptured ectopic pregnancy with a serum amylase level of 1,600 Somogyi units has been reported (Kelley, 1957).

3. DISCUSSION

The interpretation of a raised serum amylase level is complicated by the occurrence of abnormally high levels in acute abdominal conditions other than acute pancreatitis. It would clearly be of assistance if the range of values found in these extrapancreatic conditions could be defined so that the common ground shared by these and acute pancreatitis could be delineated as a zone within which caution in interpretation must be observed and possibly a line found beyond which acute pancreatitis could be diagnosed with confidence.

Bockus and Raffensperger (1948) stated that "values for amylase below five times the normal may possibly be the result of acute abdominal processes not originating in the pancreas." Probstein and Pareira (1952) considered that non-pancreatic conditions (renal retention excluded) seldom gave rise to levels over 1,000 Somogyi units and that "blood diastase levels of 1,500 units or more may be considered as pathognomic of acute pancreatitis."

In my series, while elevations of serum amylase were present in one out of every ten cases presenting with acute abdominal symptoms considered not to be pancreatic in origin, they were

all less than three times the upper limit of normal and in no case approached the levels characteristic of acute pancreatitis. Raffensperger (1951), Holt (1954) and McCollum (1955) have recorded similar experiences.

Kovacs et al. (1955) studied the serum amylase in 300 surgical patients of whom 271 had non-pancreatic conditions. Out of these 271 cases only five (1.8 per cent) had serum amylase levels above the normal range. Two cases of penetrating duodenal ulcer and two cases of intestinal obstruction had elevations which were less than five times the upper limit of normal; the fifth case was of a large pyloric ulcer apparently about to perforate and had a serum amylase level of 1,160 Somogyi units.

At first sight it would seem difficult to correlate these findings with the contrasting experience of Wilson and Seabrook (1953). They reported ten cases with serum amylase levels between 348 and 1,549 Somogyi units, of whom only three were found to have acute pancreatitis. There were also five cases of cholelithiasis, of whom three had serum amylase levels of over 1,000 units. In two of these cases the pancreas was pronounced abnormal on palpation. The two remaining cases were (1) a prepyloric ulcer penetrating the pancreas and (2)

a perforated duodenal ulcer with generalised peritonitis (1,454 units). Burnett and Ness (1955) studied 350 consecutive cases of acute abdominal disease and found that among the 336 cases with extrapancreatic disease 140 (41.7 per cent) had serum amylase levels "distinctly above normal." The majority of these were minor elevations but values over 1,000 units were found in four cases - perforated peptic ulcer (2) and intestinal obstruction (2).

Among the extrapancreatic conditions to be distinguished from acute pancreatitis, acute gastroduodenal perforation is the most important - and yet most difficult because of its close resemblance to acute pancreatitis. Paxton and Payne (1948) found in 307 cases of acute pancreatitis that the admitting diagnosis in 16.3 per cent was perforated peptic ulcer.

Probstein, Wheeler and Gray (1939), Wapshaw (1949), Malinowski (1952), Wilson and Seabrook (1953) and Pollock (1959) have each reported one case of gastroduodenal perforation masquerading as pancreatitis in which clinical error was perpetuated by the high serum amylase present and the diagnosis was finally revealed only at autopsy. In the first two cases the serum amylase levels were below 1,000 Somogyi units. Such values would now, with increased experience of serum amylase

tests and knowledge of their limitations, be treated with circumspection. In the other cases the serum amylase levels were 2,000 and 1,454 and 1,300 Somogyi units. (Kovacs et al. (1955) reported a case with a serum amylase of 1,160 units who had at autopsy a leaking chronic pyloric ulcer but it is not clear whether the ulcer was in fact perforated at the time of the blood examination). Other cases of gastroduodenal perforation with very high serum amylase are the two reported by Burnett and Ness (1955) with values of 1,600 and 3,400 Somogyi units.

The duration of symptoms correlated with the serum amylase level may sometimes give a clue to the differential diagnosis. In acute pancreatitis the serum amylase usually reaches a maximum in the first few hours after onset whereas in gastroduodenal perforation rises are unusual in the first six hours and are maximal after 12 hours (Mahaffey et al., 1955). This is not entirely reliable, as is seen in Burnett and Ness's case of gastric perforation, in which a level of 3,400 Somogyi units was reached seven hours after perforation. Complete data are not available but it would seem that in most cases of perforated ulcer with very high amylase values, the perforation had been

present for some time and there was a well-established peritonitis.

In general about a fifth of the cases of gastroduodenal perforation will show slight to moderate rises in the serum amylase. Values exceeding five times the upper limit of normal are rare but have occurred ih peptic ulcers which have perforated for seven or more hours. This serves to re-emphasise the point that, when the diagnosis is in doubt initially or becomes insecure in the course of treatment, laparotomy is indicated and a serum amylase result in the range usually associated with pancreatitis constitutes no contraindication.

As has already been stated, between a half and one-third of all cases of biliary tract disease show at some stage a raised serum amylase level and in half of these there will be intraperitoneal evidence of pancreatitis. It is held that, where the serum amylase is significantly raised in these cases, biliary-pancreatic derangement is present.

Certain extra-abdominal conditions may produce hyper-amylasaemia. Acute suppurative or acute obstructive parotitis may raise moderately the serum amylase but values over 1,000 mg. per cent occasionally occur. In these cases the cause of the raised serum amylase is usually self-evident but epidemic

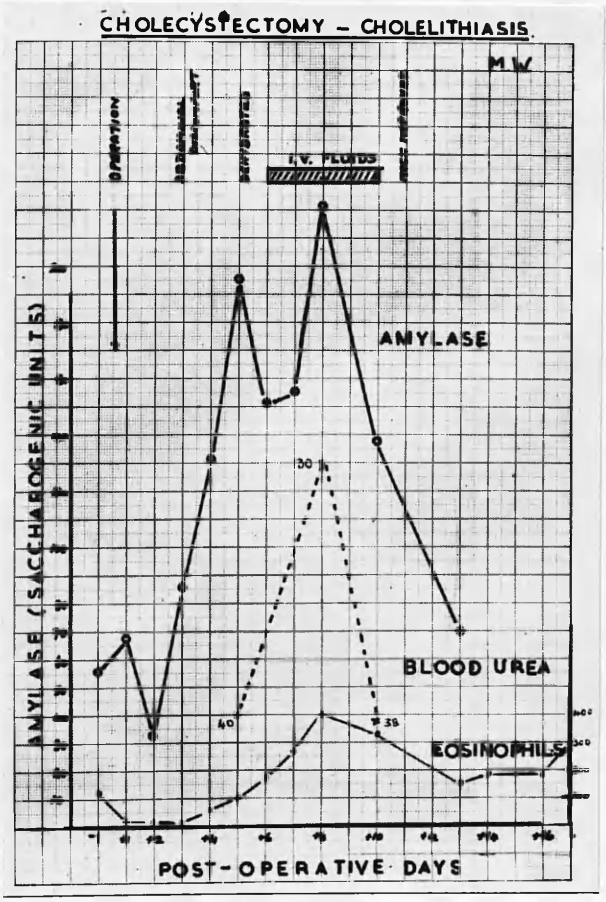
parotitis or mumps, in view of its sometimes atypical manifestations and abdominal complications, merits more attention. Hyperamylasaemia is present in about 80 per cent of cases of mumps (Candel and Wheelock, 1946; Warren, 1955) and it may be present in the absence of parotid swellings, as in submandibular mumps. Abdominal pain and colic is frequent in mumps and acute pancreatitis occurs as a complication in about 2.4 per cent of cases (Brahdy and Scheffer, 1931).

Finally, it should again be mentioned that renal retention for amylase may be a cause of hyperamylasaemia. Raffensperger (1951), in a case of intestinal obstruction with a raised serum amylase, noted that there was also a rise in the blood urea and considered that renal failure was contributory to the hyperamylasaemia. I have observed a similar renal insufficiency following cholecystectomy. A rise in the serum amylase was accompanied by a rise in the blood urea level and blood levels rapidly returned to within normal range after correction of the dehydration (Fig. 4).

Apart from Raffensperger's case, there has been little mention of renal function in the cases reported in the literature of hyperamylasaemia associated with acute extra-pancreatic abdominal conditions. In any atypical case of hyperamylasaemia, it would seem wise to examine the renal function, especially for excretion of amylase.

Figure 4.

Defective Urinary Excretion contributing to Hyperamylasaemia



PART THREE

SERUM AMYLASE IN EXPERIMENTAL PANCREATITIS AND THE EFFECT PRODUCED BY SOME THERAPEUTIC SUBSTANCES

1. PRODUCTION OF OBSTRUCTIVE PANCREATITIS

   (a) Introduction

   (b) Method

   (c) Normal Serum Amylase Levels in the Rat

   (d) Effects of Obstructive Pancreatitis in the Rat

   (e) Influence of Biliary Reflux in Obstructive Pancreatitis in the Rat

2. INFLUENCE OF DRUGS ON COURSE OF PANCREATITIS

   (a) Ganglion-blocking Agents:
      (i) Propantheline Bromide ("Probanthine")
      (ii) Hyoscine N-butyl Bromide ("Buscopan")

   (b) Cortisone

   (c) Propylthiouracil

   (d) Acetazolamide ("Diamox")

3. SUMMARY AND CONCLUSIONS

1. PRODUCTION OF OBSTRUCTIVE PANCREATITIS

(a) Introduction

When the pancreas is damaged by acute disease or injury, excessive amounts of pancreatic enzyme are released into the interstitial tissues, whence they are absorbed into the circulation or seep into the retroperitoneal tissues. These enzymes are liable, under circumstances still ill-defined, to produce further destruction of the pancreas and to have profound systemic effects. Accordingly much of the treatment of acute pancreatitis has been directed towards reducing the secretion of the pancreas and thus preventing further tissue autolysis and destruction. Several of the drugs used for this purpose have appeared to have a beneficial effect upon the course of the illness but a survey of the literature indicates that, in some cases at least, the evidence that this has been due to an effective suppression of the pancreatic secretion is rather inconclusive.

I have made studies of the effects of certain therapeutic substances upon the pancreas of the rat, both in the absence and presence of pancreatic duct obstruction, with special

emphasis on their effects upon the amount of amylase circulating in the blood. All of the drugs chosen have some claim as inhibitors of pancreatic function and secretion, though the mode and site of action upon the gland vary.

It has been known for some time (Wohlgemuth, 1909) that simple ligation of the pancreatic duct in the experimental animal is followed by swelling and oedema of the pancreas, the appearance of a variable amount of intraperitoneal fat necrosis, and a marked and significant rise in the serum amylase. When the duct and ductules become dilated and the acinar cells ectatic, secretion ceases and the serum amylase falls to subnormal levels. The remaining amylase in the blood is of extra-pancreatic origin. In some animals the changes following duct ligation are more pronounced when the pancreas is at the same time stimulated to maximal secretion by food, secretin or Mecholyl (Lium and Maddock, 1948), but this does not appear to be so in the rat (Block, Wakin and Baggenstoss, 1954).

The "obstructive pancreatitis" developing as a result of pancreatic duct ligation is, in most instances, a non-lethal lesion resembling interstitial pancreatitis as encountered in

the human (Shingleton, Anlyan and David, 1952).

In most experimental work on pancreatitis it has been considered desirable to use an animal preparation with haemorrhagic or necrotic pancreatitis for therapeutic trials. Many techniques have been devised to produce necrotic pancreatitis in animals but all have the disadvantage that the methods of production are quite unnatural and the results obtained are too variable to allow anything except a comparison of mortality rates between two large series of animals (Thal, 1954; Anderson et al., 1958). Moreover, in haemorrhagic or necrotic pancreatitis, whether naturally occurring or experimentally produced, many factors, including blood loss, infection, and electrolyte disturbances, affect the course of the disease and may contribute materially towards a fatal outcome. It seemed more appropriate in this investigation to use animals with "obstructive pancreatitis" produced by duct ligation, in which these other factors play a much less important part.

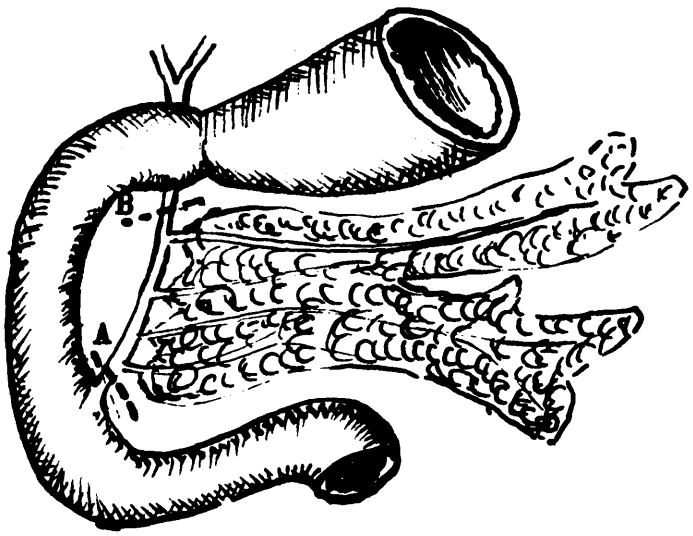
(b) Method

(i) Rat Preparations Used. In these experiments, male albino rats (Wistar strain), weighing between 250 and 350 Grams were used. Male rats only were used as Tuba and Wiberg (1953) found that the normal serum amylase levels in the two sexes were significantly different. Throughout the experiments drinking water and food were unrestricted.

In the rat, the pancreas is a diffuse structure located mainly in the mobile mesentery of the duodenum with multiple pancreatic ducts entering into a common biliary-pancreatic duct (Fig. 5). These ducts are small, friable and variable in number and position so that effective obstruction to the pancreatic duct system requires ligation of the main biliary-pancreatic duct distally. When this is done, obstructive jaundice is inevitable and there is a progressive distension of the ducts with a rising death rate amongst the rats after the third day due to rupture of the bile ducts. Therefore, these rat preparations were regarded as having an useful life of not more than four days, and observations were limited to within this period. Three rat preparations were used:

Figure 5.

Biliary-Pancreatic Duct Systems in the Rat.



Tie at A only - Connection between biliary & pancreatic duct systems

Tie at A & B - No connection between biliary & pancreatic duct systems

Preparation A: The common biliary-pancreatic duct was divided between ligatures at the duodenum. Obstruction was thus produced to both the biliary and pancreatic duct systems with anatomical continuity remaining between them so that regurgitation from one duct system to the other remained possible.

Preparation B: In this preparation a further ligature was placed round the common bile duct below the junction of the hepatic ducts and above the level of entry of the pancreatic ducts. By this means, isolated obstruction to the biliary and pancreatic duct systems was produced, and no regurgitation of bile into the pancreatic ducts was possible.

Preparation C: In a small series of rats, the common bile duct was ligated above the level of entry of the pancreatic ducts, thus producing, for control purposes, obstruction to the biliary duct system without any pancreatic duct obstruction.

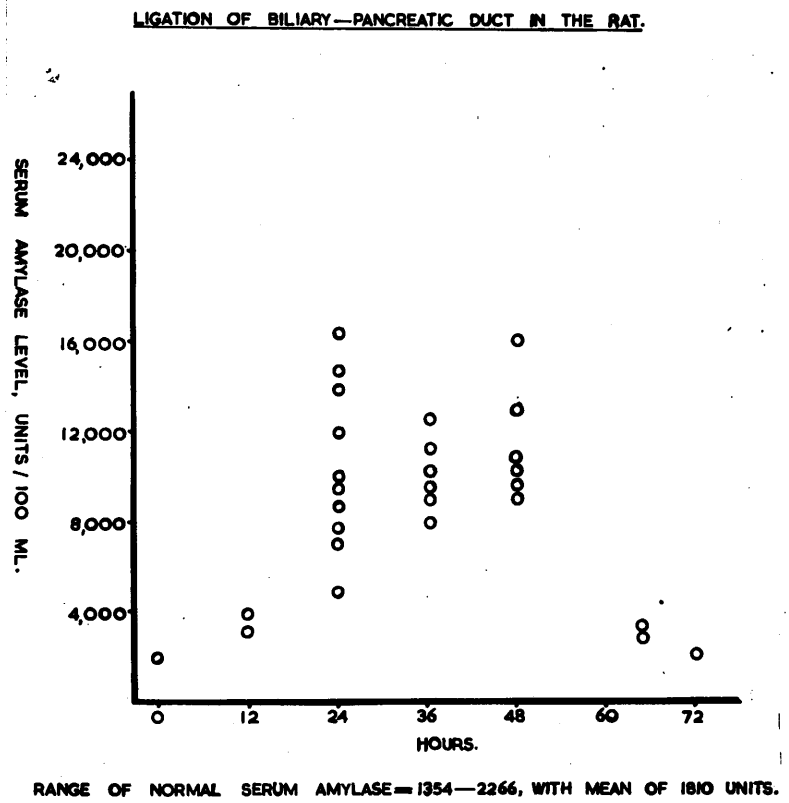
(ii) Biochemical Methods Used. The method used for the estimation of the serum amylase was Tallute's (1954) modification of Somogyi's Saccharogenic Method (1938). For

values below 3,000 units/100 ml., a 1:10 dilution of serum in normal saline was used, and for values above this level the test was repeated with a 1:100 dilution. To stabilise the pH of the test solution at these dilutions within the range of optimal enzyme activity, 2 ml. of phosphate buffer (pH 7.0) was added, as recommended by Van Loon, Likins and Seger (1952).

Adequate blood samples for serial estimations of amylase content were not available from these rats and only one measurement was made from each rat. At the appointed time therefore blood was withdrawn by direct cardiac puncture and the rat was then sacrificed and examined. In Fig. 6, which follows, each circle represents the serum amylase level in a rat which was examined at the post-ligation time indicated on the abscissa and the composite picture is made up from the results in 28 rats. Thus there is no direct measurement of the rise and fall of the serum amylase during the course of the experiment. This is true of all the subsequent charts in this section.

Figure 6.

Serum Amylase Levels after Ligation of the Biliary-Pancreatic Ducts in Rats



(c) Normal Serum Amylase Levels in the Rat

Serum amylase levels were measured in 25 normal rats, using the biochemical method described above. Readings ranged from 2290 to 1504 units/100 ml., with an Arithmetic Mean of 1810 units/100 ml.. The Standard Deviation was 228 units/100 ml., and the Coefficient of Variation 12.6. (For readings obtained see Appendix C.2).

(d) Effects of Obstructive Pancreatitis in the Rat

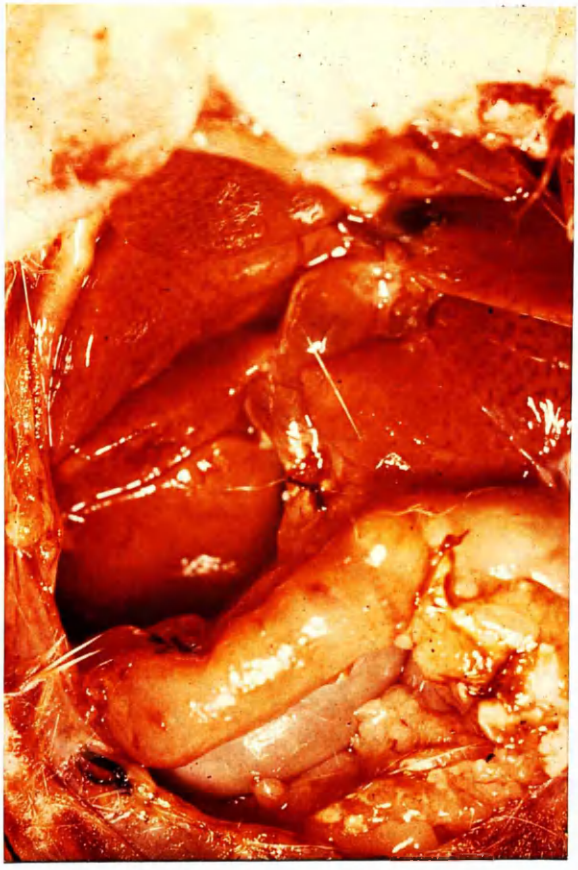
Preparation B, in which the segment of the common biliary-pancreatic duct draining the pancreatic ducts was isolated by proximal and distal ligatures, was used in 21 rats. Twenty rats surviving the operation were killed in batches at 24, 36, 48 and 72 hours after ligation.

During the first 24–48 hours after the operation, the rats became jaundiced, were listless, bedraggled and dirty and usually showed some abdominal distension. Thereafter they improved in appearance and appeared more lively, though the urine remained heavily loaded with bile. Laparotomy showed progressive dilatation of the biliary and pancreatic ducts, with the pancreas swollen and oedematous but not stained with bile (Fig. 7a). At 24 hours or so, there was usually some ileus and by 48 hours a variable amount of fat necrosis (Fig. 7b).

The serum amylase levels attained are presented in detail in Appendix C.3 and are analysed in Table XIII. It will be observed that the serum amylase values followed a well-defined pattern with high readings, between $2\frac{1}{2}$ and 9 times the normal range, at 24, 36 and 48 hours after duct-ligation. The results in this experiment are shown graphically in Fig. 8.

Figure 7.

Intraperitoneal Appearances in Obstructive Pancreatitis in the Rat.



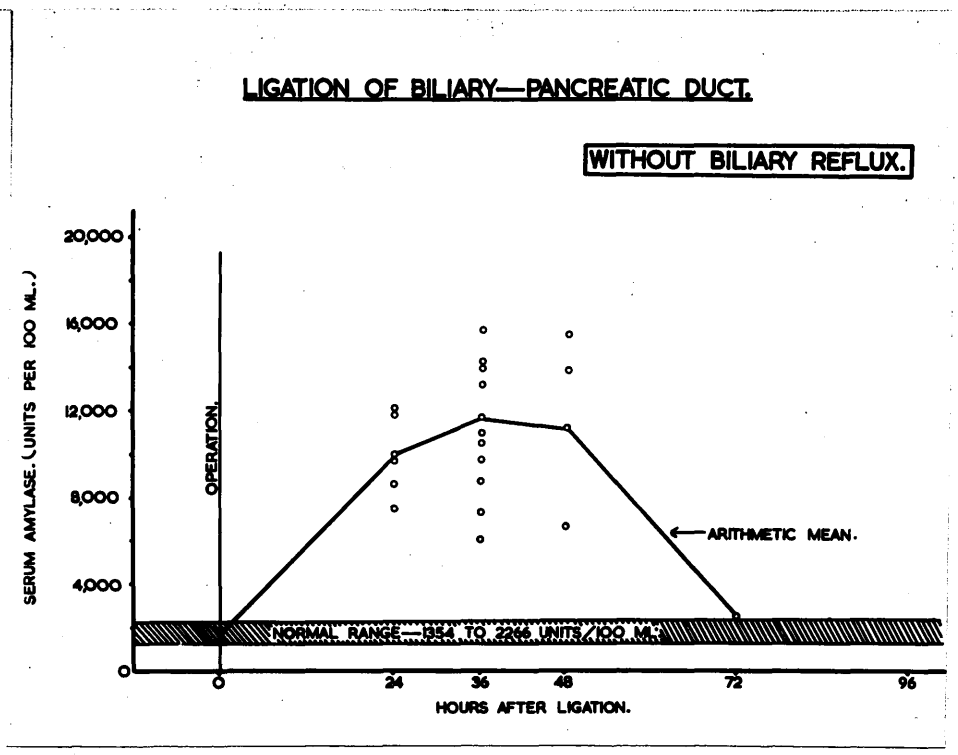
(a) Distended bile ducts 48 hours after ligature. Note the double distal ligature and single proximal ligature.

(b) Fat necrosis in mesentery.

TABLE XIII. Ligation of Biliary-Pancreatic Duct without Biliary Reflux

	Post-ligation Serum Amylase (Units/100 ml.)			
	24 hours	36 hours	48 hours	72 hrs.
Range	12,110-7,654	17,704-6,182	16,439-6,670	2,343
Mean	10,000	11,700	11,400	2,343
S.D.	1,992	3,614	-	-
Number of Rats	5	11	3	1

Figure 8


LIGATION OF BILIARY—PANCREATIC DUCT.
WITHOUT BILIARY REFLUX.
SERUM AMYLASE. (UNITS PER 100 ML.)
20,000
16,000
12,000
8,000
4,000
0
OPERATION.
ARITHMETIC MEAN.
NORMAL RANGE—1354 TO 2246 UNITS/100 ML.
0
24
36
48
72
96
HOURS AFTER LIGATION.

In order to exclude biliary duct obstruction as a significant contributing cause of the marked hyperamylasaemia obtained, the bile duct was ligated, in a small control group of rats, above the level of entry of the pancreatic ducts so that there was biliary obstruction without pancreatic obstruction (Preparation C). This produced no marked rise in the serum amylase levels (Table XIV).

TABLE XIV. Serum Amylase Levels in Simple Biliary Obstruction

Hours after Duct Ligation			
27	38	45	66
(1) 1,592 (2) 1,517	(3) 1,762 (4) 1,518	(5) 3,500 (6) 2,581	(7) 1,953

Readings taken from 7 rats sacrificed at above times.

(e) The Influence of Biliary Reflux in Obstructive Pancreatitis in Rats.

(i) Introduction. In 1901, Opie reported a fatal case of acute haemorrhagic pancreatitis in which he had found at autopsy a gallstone impacted in the ampulla of Vater, thus diverting bile into the pancreatic duct. He postulated that the irritant bile devitalised the parenchymatous, vascular, and interstitial tissues of the pancreas so that they became vulnerable to the digestive ferments of the pancreatic juice, notably trypsin. Later (1903) he published further clinical and experimental evidence to support his theory. The association of pancreatic disease and disease of the bile passages, especially cholelithiasis, had already been noted by a number of writers (Korte, 1898; Lancereaux, 1899) but the general view was that stagnation in the ducts had facilitated spread of infection from the bile duct to the gland.

Subsequent investigators have found that a small Vaterine stone was present in only about five per cent of cases of acute pancreatitis (Schmieden and Sebening, 1927; Ivy and Gibbs, 1952) but interest in the "Common Channel" theory of the aetiology of pancreatitis has persisted with spasm, oedema or fibrosis as other possible causes of obstruction at the

duodenum (Archibald, 1919).

Acute pancreatitis, however, is not always dependent upon the presence of a "Common Channel" as it also occurs in the absence of pancreatic duct obstruction, biliary disease, or biliary reflux into the pancreatic ducts. Opinions as to the importance of the "Common Channel" in the aetiology of acute pancreatitis vary considerably.

In spite of a great deal of study by many workers, there is little agreement as to the incidence of an anatomic common channel. Ivy and Gibbs (1952), in a wide survey of the literature, found that the reported incidence varied from 16 to 64 per cent with an overall incidence in the collected series of 32 per cent. Sterling (1954) made an anatomical study of the ampullary region in 1,252 cadavers and came to the conclusion that a functioning common biliary-pancreatic channel which would permit interductal reflux was present in about 15 per cent of the population. In studies made upon living persons by observing the frequency of filling during cholangiography the results between the various reported series range from 9 per cent to 41 per cent with an overall average, in a total of 1,434 observed cases, of 35 per cent. Thus biliary reflux into the pancreatic tree would appear to be a possibility in at least a third of persons.

Bile does not necessarily flow into the pancreatic tree when there is an obstruction distal to a common biliary-pancreatic channel, The normal secretory pressure of the pancreas is higher than that of biliary secretion (Cattell and Warren, 1953), and the frequent finding of pancreatic ferments in the gall bladder (Popper, 1933) would support the view that in the presence of ampullary obstruction there is a flow of secretion from the pancreatic duct system to the bile ducts.

The work of Herring and Simpson (1909) and Elliott, Williams and Zollinger (1957) would indicate that with obstructed, but communicating, biliary and pancreatic duct systems the hydrodynamics present is even more complicated. Herring and Simpson observed that, when the pancreatic duct was obstructed in certain experimental animals, the pressure of the pancreatic secretion rose to a level closely approximating to that reached by the bile in obstruction of the common bile duct, the rise and fall of the pancreatic secretory activity being more rapid than the rise and fall of the bile pressure. This was confirmed by Elliott, Williams and Zollinger, who have shown that (in the dog) there is a tidal flow of the pancreatic secretions into the biliary tract and then, after 24 hours or so of obstruction, there is a reflux of

a mixture of bile and pancreatic secretions (with the trypsin now activated) into the pancreatic ducts.

The mere presence of bile in the pancreatic ducts has little harmful effect (Whitrock, Hine, Crane, and McCorkle, 1955) and the consistent production of necrosis of the pancreas by the injection of bile, as in Opie's experiments, has been achieved only by the use of injection pressures considerably higher than those occurring normally (Rich and Duff, 1936). Elliott, Williams, and Zollinger (1959), in the experiments previously mentioned, have shown that, when bile and pancreatic juice have been incubated together for 12 - 24 hours are introduced into the pancreatic duct at a physiological pressure (40 cm. water), large amounts readily permeate into the pancreatic gland to produce severe haemorrhagic pancreatitis. They concluded that in the natural disease the incubation of bile and pancreatic juices took place in the early phase after a common channel obstruction when, due to the higher pancreatic secretory pressure, pancreatic juice flowed up the biliary ducts into the gall bladder; and that haemorrhagic pancreatitis occurred when, after pancreatic secretion was inhibited, the flow was reversed. They found experimentally that a critical quantity

of bile and pancreatic secretion, near equal quantities, were necessary both for entry of the mixture into the pancreas at low pressure and for true haemorrhagic pancreatitis to result. It may be that biliary pancreatitis can vary widely in severity from mild oedema to overwhelming necrosis depending upon the constituents of the solution infiltrating the pancreas.

The most generally accepted theory concerning the action of bile is that it acts as an activator of trypsinogen (Opie, 1903; Wangensteen et al, 1931; Rich and Duff, 1936; Dreiling and Richman, 1954). It is doubtful if the activation of trypsinogen alone is sufficient to trigger off the explosive onset of haemorrhagic pancreatitis. Elliott et al (1958) have shown that, when bile or trypsin are perfused at physiological pressures into the pancreatic ducts of dogs, little harm is done but the perfusion of trypsin and bile produces a haemorrhagic pancreatitis. Thus it is likely that in "bile" pancreatitis the bile not only activates the trypsinogen but makes the gland more vulnerable to the enzyme onslaught, either by a direct devitalising action or by some effect upon the vasculature. In support of the latter mechanism is the finding of Thal (1954) that interstitial bile markedly reduces local blood flow.

It would appear from these observations that biliary reflux into the pancreatic ducts might facilitate the progression of an interstitial pancreatitis produced by pancreatic duct obstruction into acute pancreatic necrosis.

(ii) Experiment. In a group of 26 rats the common biliary-pancreatic duct was tied distally only (Preparation A), so that the regurgitation of bile into the pancreatic tree was an anatomical possibility. These animals were killed at 24, 36, 48, 72 and 96 hours.

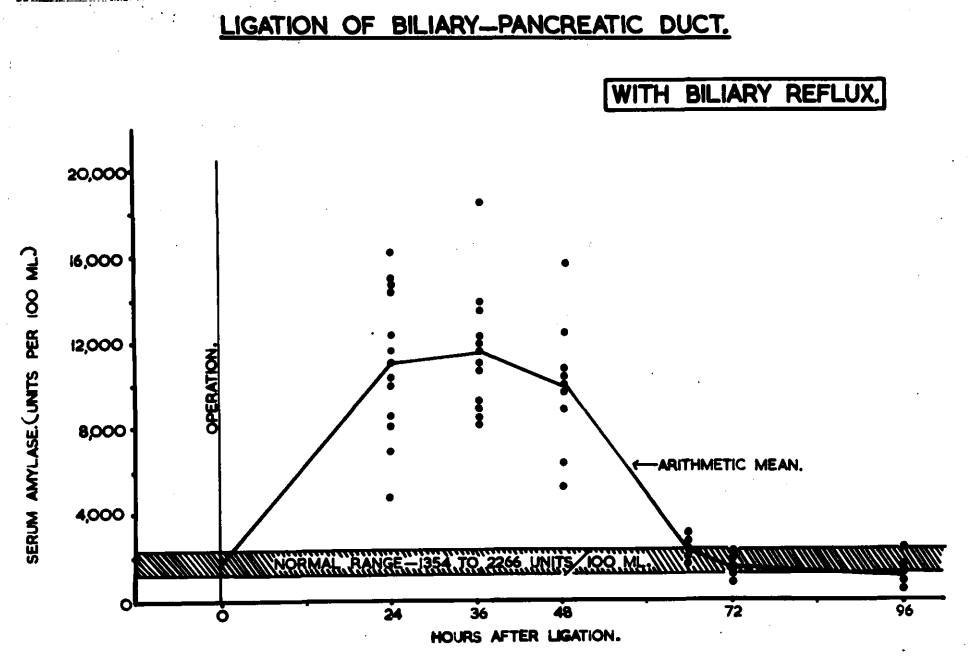
(iii) Results. There was visible bile-staining of the distended pancreatic ducts in the later stages but apart from this the intra-peritoneal and general appearances were the same as when obstruction had been produced without biliary regurgitation. The serum amylase results are analysed in Table XV and are presented in detail in the appendix (Table C.4). The post-ligation trend in the serum amylase showed a pattern identical to that found in the previous experiment without any significant difference between the levels in the corresponding groups (Fig. 9).

(iv) Conclusion. In these rat experiments the reflux of bile into the pancreatic ducts, which was visible both macroscopically and microscopically, failed appreciably to alter the course of the experimentally induced obstructive pancreatitis.

TABLE XV. Serum Amylase in Obstructive Pancreatitis with Biliary Reflux

	Post-Ligation Serum Amylase (units/100 ml.)				
	24 hrs.	36 hrs.	48 hrs.	72 hrs.	96 hrs.
Range	16,236-4,876	18,492-7,945	15,798-5,252	2,358-1,240	1,824-784
Mean	11,000	11,530	9,890	1,710	1,283
S.D.	3,584	3,039	3,154	543	436
No. of Rats	12	11	8	4	4

Figure 9.


LIGATION OF BILIARY–PANCREATIC DUCT.
WITH BILIARY REFLUX.
SERUM AMYLASE. (UNITS PER 100 ML.)
20,000
16,000
12,000
8,000
4,000
0
OPERATION.
←ARITHMETIC MEAN.
NORMAL RANGE — 354 TO 2266 UNITS / 100 ML.
0
24
36
48
72
96
HOURS AFTER LIGATION.

2. INFLUENCE OF DRUGS ON COURSE OF PANCREATITIS

(a) Ganglion-blocking Agents

(i) Introduction. In the treatment of acute pancreatitis with atropine or the newer ganglion-blocking agents, the effect desired is to produce a chemical "vagotomy." As a result of Pavlov's work on pancreatic secretion in the dog it has been accepted that stimulation of the vagus results in an increased secretion of pancreatic enzymes and from this it has been assumed that the "enzyme content of pancreatic juice is determined by the vagus nerve" (Mellanby, 1925). It seems likely, however, that the vagi are not the only sources of secretomotor stimuli reaching the pancreas and that there is a peripheral reflex mechanism which receives stimuli initiated locally as well as stimuli transmitted from the central nervous system by the vagus nerves (Thomas and Crider, 1944).

It is less well-known how far pancreatic secretion is affected by abolition of vagal activity. Routley et al (1950) found, as a result of their animal experiments, that vagotomy produced no statistically significant disturbance in external pancreatic function, which was in agreement with the earlier investigations of Thomas and Crider (1944). Thistlethwaite (1951) found that vagotomy in dogs (excluding the immediate

postoperative phase) caused a reduction of about 20 per cent both in amylase content and in the volume of the pancreatic secretion.

In man, Shingleton, Fawcett and Vetter (1950) found that, after vagotomy, the average resting volume of the pancreatic secretion was higher than normal but that there had been produced a complete blockage of response to intravenous secretin. Pfeffer, Stephenson and Hinton (1952) reported that, following vagotomy, the enzyme output and total volume of pancreatic secretion was reduced by almost two-thirds but it should be noted that during these investigations the pancreas was maintained in a state of continuous maximal secretion by means of intravenous secretin.

A similar pattern of response has been obtained through "chemical vagotomy" with ganglion-blocking agents (Thistlethwaite, 1951; Annis and Hallenbeck, 1950; Shingleton et al., 1950; and others). Pfeffer and Hinton (1950), using duodenal drainage and maximal stimulation of pancreatic secretion with secretin and insulin, found that "Probanthine" reduced the volume of pancreatic juice 52.6 per cent, bicarbonate concentration 26.4 per cent and significantly reduced the amylase output in six out of ten patients tested. Sinclair (1956) and Boba and Korkosz (1957) found "Probanthine" effective in the treatment of external

pancreatic fistulae with marked reduction in the volume of the secretion.

From these findings it would appear that vagotomy has little or no effect upon the resting pancreatic secretion and that its inhibiting effect is marked only in the activated pancreas. It is effective in blocking the water, bicarbonate and enzyme response of the pancreas to intravenous secretin (Shingleton et al., 1950), hypoglycaemia (Babkin, 1950; Routley et al., 1950; Dreiling et al., 1952), peptone, hydrochloric acid and food in the upper intestine (Thomas and Crider, 1944; Annis and Hallenbeck, 1950) and psychic stimuli (Routley et al., 1950).

It seems from these reports also that the effect of ganglion-blocking agents upon pancreatic secretion may be greater than that obtained by surgical vagotomy. Possibly these substances have a more widespread blockading action upon the peripheral reflex mechanisms than can be achieved by an operation limited to dividing the long association pathways from the central nervous system. In the literature concerning the effects of vagotomy upon the course of pancreatic disease, reports are also conflicting. Schafferzick et al. (1951) found that, when the pancreatic ducts were tied in previously vagotomised dogs, the resultant rise in the serum amylase was less

pronounced and less sustained than in dogs with their vagi intact, though the difference was statistically significant only from the third post-ligation day onwards. The accompanying microscopic changes in the pancreas varied in degree, inflammatory cell infiltration, haemorrhages, acinar necrosis, and fat necrosis being on the whole more marked in the control series. In their experiments stimulation of the pancreatic secretion was maintained after ligation of the pancreatic ducts by tube-feeding the dogs with evaporated milk.

Khedroo (1957) produced experimental haemorrhagic pancreatitis in dogs by ligation of pancreatic ducts after retrograde injection of bile-trypsin mixture, and found that vagotomy had no effect upon the mortality, the severity of the acute pancreatitis or the serum amylase levels. Kusunoki (1936) also found that vagotomy had no effect upon the course of experimental pancreatitis.

More dramatic results were obtained by Shingleton et al. (1952) using "Banthine" as a vagal-blocking agent. They used two dog preparations. In the first, oedematous pancreatitis was induced by ligation of pancreatic ducts and pancreatic stimulation with intravenous pilocarpine and secretin; and in the second acute pancreatic necrosis was induced by ligation

of pancreatic ducts and retrograde injection of bile-trypsin mixture. In the former group, treatment with Banthine reduced the post-operative rise in the serum amylase by nearly two-thirds and the reactive changes in the pancreas were less marked; in the latter group Banthine reduced the mortality from seven out of ten to three out of ten and the mean serum amylase levels were reduced by about a quarter compared with the control dogs.

It appears from these reports that the blocking of vagal impulses, whether by surgical or chemical means, has in general surprisingly little effect upon the course of experimental pancreatitis whether measured by control of hyperamylasaemia, pancreatic changes or crude mortality rates. When effective responses were obtained, as in the experiments of Schaffarzick et al. (1951) and Shingleton et al. (1952), the animals used were dogs in which maximal pancreatic secretion following ligation of the pancreatic ducts was maintained by tube-feeding or the injection of secretagogues.

(ii) Experiments. I have studied the effects of two ganglion-blocking drugs upon pancreatic secretion in the rat, both in the presence and absence of pancreatic duct obstruction. The drugs studied are reputed to differ in their mode of action.

They are:

A. Propantheline Bromide - "Probanthine" (C. D. Searle & Co.);

B. Hyoscine N-butylbromide - "Buscopan" (C. H. Boehringer Sohn).

A. Propantheline Bromide - "Probanthine"

1. Introduction. "Probanthine" is the methobromide salt of a quarternary amine (2-diisopropyl amine ethyl xanthene-9-carboxylate methobromide). It has a potent anti-cholinergic action, being 2-4 times as active as atropine sulphate in producing neuro-effector blockade at the para-sympathetic terminations. In larger doses it produces a ganglionic blockade, sympathetic and parasympathetic (Schwartz et al., 1953). Thus the principal site of action is the parasympathetic system but it exerts a single and lesser action upon the sympathetic system.

2. Method. White Wistar rats of approximately 250 G. in weight were again used. In the first group of experiments, six rats were injected with "Probanthine" 0.5 mg. twice daily (4.0 mg./Kg. body weight/day) intramuscularly. This dose of 4.0 mg./Kg. body weight twice daily was that found, after trial, to give the maximum response without signs of toxicity. The

serum amylase levels were measured after 48 hours. In the second group of experiments, the rats had their common biliary-pancreatic ducts tied at the duodenum in the manner already described. They also were given "Probanthine" in the above dosage and groups of rats were sacrificed at 24, 36, 48, 72 and 96 hours.

3. Results: (a) Effect of "Probanthine" on the Normal Rat. The rats tolerated the dosage of 4.0 mg./Kg. body weight without any apparent ill-effects. In Table XVI are given the serum amylase levels found in the six rats after 48 hours of "Probanthine" and the results are analysed. "Probanthine" caused a significant depression of the serum amylase (using the t-test, $P < 0.01$) compared with the control series.

TABLE XVI. Effect of "Probanthine" on Serum Amylase in Albino Rats (Wistar Strain)

Dose:	"Probanthine" 0.5 mg. b.i.d. (4.0 mg./Kg. body weight/day)
Number of Rats:	6
Readings Obtained:	1,740; 1,368; 1,290; 1,266; 1,189; 1,180
Mean:	1,340 units/100 ml.
Standard Deviation:	208 units/100 ml.
A.M. $\pm$ 2 x S.D.:	1,756 - 1,124 units/100 ml.

(b) Effect of Pancreatic Duct Ligation and "Probanthine." Twenty-six rats survived their allotted time and the following are the findings in these cases:

Serum Amylase: The serum amylase levels are given in detail in Table C.5 in the appendix and the results are analysed in the accompanying Table XVII. They are also illustrated graphically in Fig. 10, where the mean values of the readings obtained are connected by a continuous line.

24, 36 and 48 hours after ligation of the duct, serum amylase readings were not lower than those found in rats not receiving "Probanthine." In fact, at these times, the readings were at a higher average level than in the control series, though the differences in the means are not statistically significant.

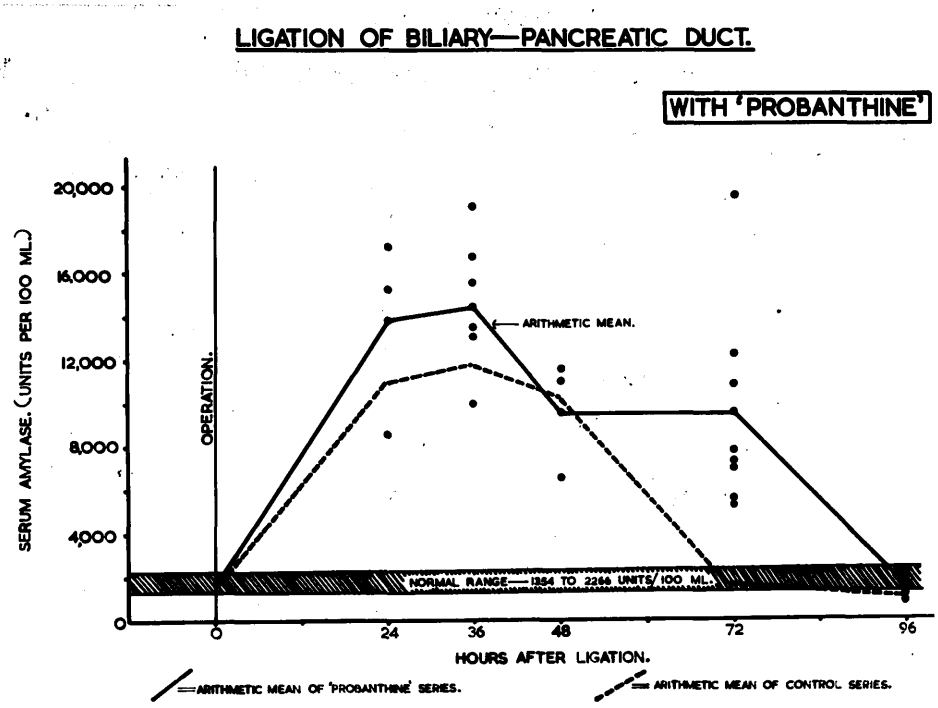
72 hours after duct ligation, the serum amylase was still markedly elevated. The range was 19,613-5,520 units with a mean of 9,468 units and a Standard Deviation of 4,700 units. There is a statistically significant difference between these results and those obtained in the control series at 72 hours ($P < 0.01$).

By 96 hours all the rats tested had serum amylase levels at normal or below normal levels.

TABLE XVII. Ligation of Biliary-Pancreatic Duct and "Probanthine"

	Post-Ligation Serum Amylase (units/100 ml.)				
	24 hrs.	36 hrs.	48 hrs.	72 hrs.	96 hrs.
Range	17,245-8,551	18,907-10,070	11,529-6,533	19,613-5,520	2,194-901
Mean	13,717	14,646	9,664	9,468	1,560
S.D.	-	3,131	-	4,706	444
No. of Rats	3	6	3	8	6

Figure 10


LIGATION OF BILIARY—PANCREATIC DUCT.
WITH 'PROBANTHINE'
SERUM AMYLASE. (UNITS PER 100 ML.)
20,000
15,000
12,000
8,000
4,000
0
OPERATION.
ARITHMETIC MEAN.
NORMAL RANGE — 1354 TO 2266 UNITS/ 100 ML.
0
24
36
48
72
96
HOURS AFTER LIGATION.
= ARITHMETIC MEAN OF 'PROBANTHINE' SERIES.
= ARITHMETIC MEAN OF CONTROL SERIES.

Other Findings. The rats in this series appeared to be more ill and to have more abdominal distension, especially in the first 48 hours, than in the control series. This distension appeared to be due to increased ileus. An arbitrary measurement of the amount of bowel distension present at laparotomy was made with grading from + to + + + (Table XVIII).

Compared with the control series, there was no difference in the appearance of the pancreas which was swollen, oedematous and sometimes visibly bile-stained in the later stages. The bile and pancreatic ducts however seemed to be less distended than those in the control series which had been obstructed for the same length of time and this was supported microscopically by the slower appearance of globules of secretion within the acinar cells. These effects were similar to those found with "Buscopan."

There appeared to be no significant diminution in the amount of fat necrosis present compared with the control series (Table XVIII).

TABLE XVIII. Effect of Ganglion-blocking Agents upon Ileus and Fat Necrosis in Experimental Obstructive Pancreatitis.

Durn. of Obst.	Control			Probanthine			Buscopan		
	Ileus	F.N.	S.A.	Ileus	F.N.	S.A.	Ileus	F.N.	S.A.
	0	++	12,271	++	++	8,551	+	0	14,848
24 hrs	+	+	10,083	±	+	15,357	0	0	14,389
	0	+	14,479	++	+	17,245	0	+	10,558
	0	+	11,241	.	.	.	0	±	15,892
	+	++	8,413	.	.	.	0	0	9,841
	+	+++	18,492	++	++	18,907	+	0	14,727
	0	0	13,891	++	+	16,804	0	++	13,124
36 hrs	0	+	13,605	++	++	15,661	0	+	11,995
	+	++	12,314	++	+	12,855	0	0	8,546
	+	+	10,942	++	+	13,571	0	+	7,957
	±	±	12,285	.	.	.	±	0	12,430
	+	++	10,445	+++	+	11,529	0	0	9,750
48 hrs	++	+	9,483	++	++	10,929	0	±	9,299
	+	++	7,486	-	+	6,533	±	±	8,882
	+	+	5,252	.	.	.	0	0	8,080
	+	+	1,288	?	+	12,227	0	±	10,242
	0	0	1,958	?	+	10,734	0	+	6,810
72 hrs	0	±	1,240	?	+	7,346	±	+++	10,042
	±	+	2,358	?	0	6,958	0	++	6,373
	0	++	2,343	?	0	5,520	0	0	8,536

S.A. Serum Amylase (mg.%)

F.N. Fat Necrosis
+ few spots
++ Localised patch
+++ Widespread
++++ Extensive both intra- and extraperitoneal

B. Hyoscine N-butylbromide - "Buscopan"

1. Introduction. "Buscopan" is a quaternary ammonium compound produced synthetically from Scopolamine. It is a ganglion-blocking agent with, it is claimed, an almost specific effect upon the ganglia of the parasympathetic chain and little or no effect upon the parasympathetic neuro-effectors.

Erlsbacher and Geisberger (1954) treated 25 patients with acute, subacute and chronic pancreatitis and claimed a good therapeutic response, especially in patients with acute pancreatitis.

2. Method. As in the previous experiments, albino Wistar rats of about 250 G. in weight were used. "Buscopan" was injected intramuscularly in a dosage of 0.4 mg. b.d. (3.0 mg./Kg. body weight/day).

(a) Six rats were given "Buscopan" for 48 hours and then the serum amylase was examined.

(b) Twenty-seven rats had the common biliary-pancreatic duct tied between ligatures distally and were given "Buscopan" in the above dosage. Rats were killed at 24, 36, 48, 72 and 96 hours and had a serum amylase estimation and general autopsy examination.

3. Results: (a) The Effect of "Buscopan" upon the Serum Amylase Secretion in Normal Rats. The results are recorded in Table XIX. The mean serum amylase level is 1,135 with a standard deviation of 207 units/100 ml. When compared with the normal mean of 1810 and S. D. ± 228, the depression in the serum amylase level is significant, P being < 0.01.

TABLE XIX. Effect of "Buscopan" on Serum Amylase in Albino Rats (Wistar Strain)

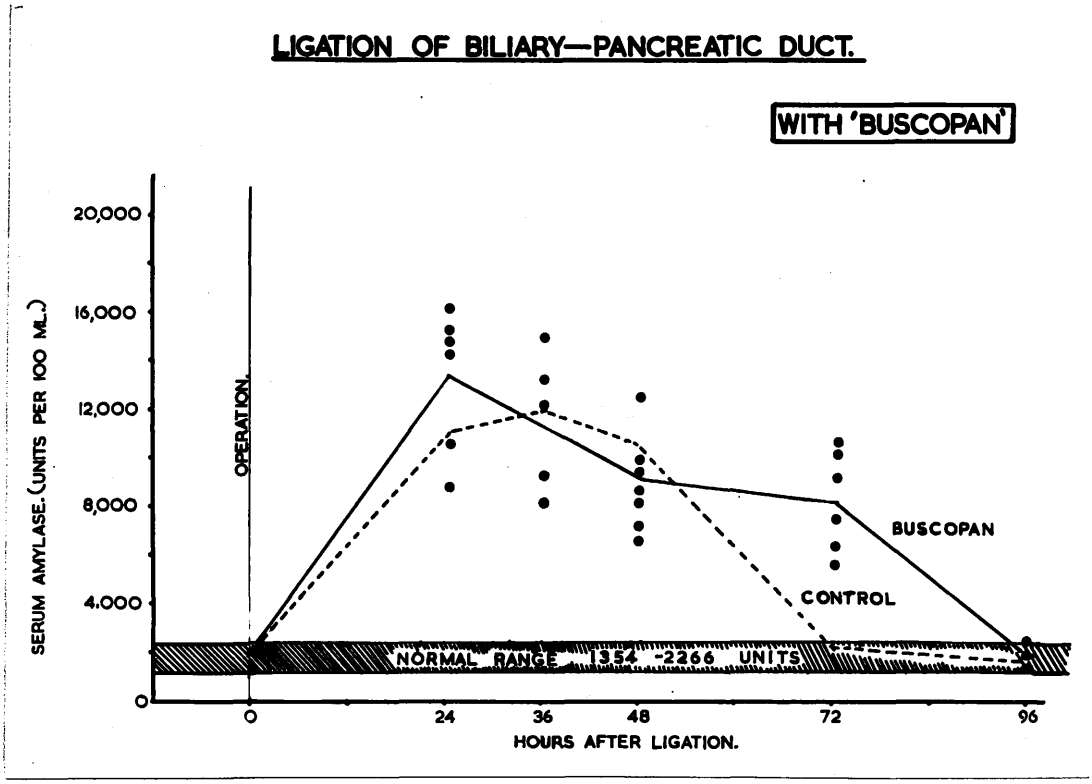
Dose:	"Buscopan" 0.4 mg. b.d. for 48 hours (3.0 mg./Kg. body weight/day)
Number of Rats:	6 of 260 G. average weight
Readings Obtained:	1,445; 1,335; 1,097; 1,023; 960; 953
Mean:	1,135 units/100 ml.
Standard Deviation:	207 units/100 ml.
A.M. - 2 x S.D.:	1,549 ± 721 units/100 ml.

(b) The Effect of "Buscopan" and Biliary-Pancreatic Duct Obstruction upon the Serum Amylase. The results obtained are given in Table XX and shown graphically in Fig. 11. It will be observed that 24, 36 and 48 hours after duct ligation, as with "Probanthine," results were within the range obtained in the control series. The serum amylase levels were still significantly elevated at 72 hours and near normal levels were not

TABLE XX. Ligation of Biliary-Pancreatic Duct and "Buscopan"

	Post-Ligation Serum Amylase (units/100 ml.)				
	24 hrs.	36 hrs.	48 hrs.	72 hrs.	96 hrs.
Range	15,892-9,841	14,727-7,957	12,430-6,945	10,242-5,626	2,008-1,328
Mean	13,343	11,269	8,906	7,938	1,775
S.D.	2,500	2,933	1,755	1,958	-
No. of Rats	6	5	7	6	3

Figure 11.


LIGATION OF BILIARY—PANCREATIC DUCT.
WITH 'BUSCOPAN'
SERUM AMYLASE. (UNITS PER 100 ML.)
20,000
16,000
12,000
8,000
4.000
0
OPERATION.
BUSCOPAN
CONTROL
NORMAL RANGE 1354 -2266 UNITS
0
24
36
48
72
96
HOURS AFTER LIGATION.

encountered until 96 hours after ligation.

The appearances of the pancreas were similar to those in the previous experiments but the following extra observations were made:

i. Degree of Duct Distension: The diameters of the distended common biliary-pancreatic ducts as found in rats 24, 48, 72 and 96 hours after distal ligation of the ducts were measured by photographing the sacrificed rats with a measure alongside the opened abdomen. The photographs were then projected on to a screen and the diameter of the biliary-pancreatic duct immediately below the lowermost hepatic duct was measured. Ten "Buscopan"-treated rats and eleven control rats were examined in this way. The mean values obtained (Table XXI) confirmed that there was less duct distension in the "Buscopan"-treated rats than in the control rats during the first four days of the experiment.

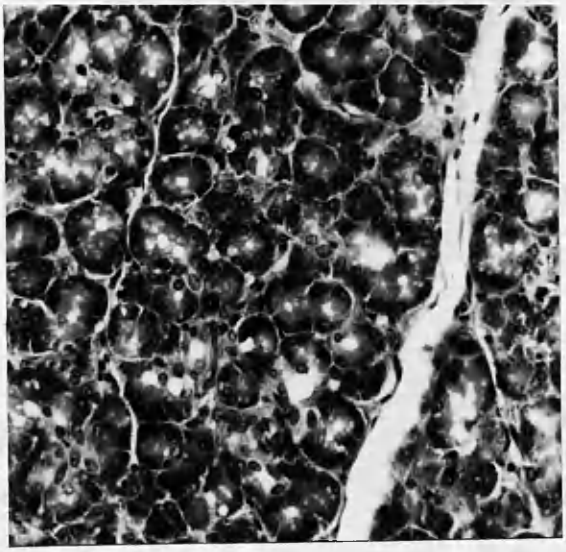
ii. Acinar Ectasia: Histological examination of the pancreas at the various intervals after duct ligation showed that there was a slower development of globules of retained secretion within the acini in the "Buscopan"-treated rats than in the controls (Fig. 12).

TABLE XXI. Diameter of Biliary-Pancreatic Duct After Ligation. (Control and "Buscopan" Series)

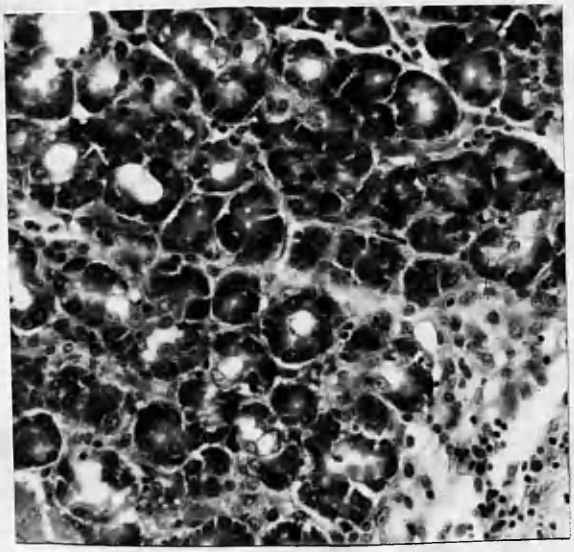
Post-Ligation Interval (Hours)	Mean Diameter B-P Duct (in mm.)	
	Control	"Buscopan"
24	2.8	1.5
48	3.9	2.8
72	4.8	4.0
96	5.6	4.8

Figure 12.

Histological Appearances of Pancreas in Buscopan-treated and Control Rats



(a) Pancreas after 48 hours obstruction in Buscopan-treated rat.
(x 225. H & E.)



(b) Pancreas after 48 hours obstruction in untreated rat.
(x 225. H & E.)

iii. Enzyme Content of the Retained Pancreatic Secretion: In several rats, fluid was aspirated from the distended ducts and measured for amylase content (Table XXII). There was no difference between the two series in the rather approximate* results obtained and there was no evidence forthcoming that the "Buscopan" had produced a selective reduction in the enzyme content of the pancreatic secretion.

Other Findings. There appeared to be less intraperitoneal fat necrosis present compared with either the "Probanthine" or control series (Table XVIII). Also it was observed that the amount of ileus present appeared to be less than in the "Probanthine" series and possibly also less than in the control series (Table XVIII).

* The aspirate on each occasion was diluted 100 times before being tested by the iodometric method.

TABLE XXII. Amylase Content of Retained Secretions after Biliary-Pancreatic Duct Obstruction (Control and "Buscopan" Series)

Duration of Duct Ligation (Hours)	Amylase Content (Iodometric Units/100 ml.)	
	Controls	"Buscopan"
24	400,000 200,000 200,000	266,000
48	66,000 73,000 80,000	80,000 100,000
72	160,000 183,000	106,000 106,000

(iii). Discussion. In the rat, dosage with either "Probanthine" or "Buscopan" depressed pancreatic secretion as measured by the level of the amylase in the blood. In spite of this apparent reduction in secretory activity of the pancreas these drugs did not reduce at all the degree of hyperamylasaemia following pancreatic duct obstruction. The duration also of the hyperamylasaemia was prolonged so that high blood levels were still present after 72 hours, whereas in the control series after the same length of time the amylase content of the blood was no longer raised.

The duct distension and acinar ectasia found in the drug-treated rats was of relatively less degree than in the control animals and this provides evidence to support the view that the hyperamylasaemia was prolonged because "Probanthine" and "Buscopan" reduced the rate of secretion in the pancreas sufficiently to delay the onset of distension destruction of the secretory epithelium.

Thus there seems in my experiments to be confirmation for the view that "Probanthine" and "Buscopan" reduce the secretory activity of the pancreas. Some explanation is however necessary to account for the persistence of a high blood amylase level during the first 48 hours after pancreatic duct

obstruction. This is due not to any increase in the total pancreatic secretion but to the diversion of a greater part of the secretion into the interstitial tissues of the pancreas. Normally the amount of pancreatic secretion passing into the blood stream is small, probably about one per cent of the total secretion, but in acute pancreatitis the permeability of the gland is profoundly altered, resulting in flooding of the interstitial tissues with secretion, sufficient to obscure the lesser changes consequent on partial reduction of the total pancreatic secretion. In the "Buscopan"-treated rats, less intraperitoneal fat necrosis was observed, indicating that there had been some reduction in the spill of enzymes not reflected in the serum amylase.

Schafferzick et al. (1951) and Shingleton et al. (1952) were able to demonstrate some reduction in the serum amylase levels in their dogs, but in their experiments (though not in my experiments with rats) maximal pancreatic secretion was artificially maintained throughout. While under these conditions an exaggerated response from "vagotomy" would be expected in a hypersecreting pancreas, the conditions are not comparable to those in clinical practice where ancillary measures to reduce pancreatic secretion are routinely employed.

The vascular state of the pancreas also plays some part in determining the level of amylase in the blood. A good blood flow facilitates the clearance of extravasated secretion with the production of a higher blood enzyme content than would be found with the same amount of extravasation but a lesser blood flow.

The only objective side-effect that was observed with the ganglion-blocking agents was the apparent increase in ileus with "Probanthine." Paralytic ileus is a well-known side-effect of certain of the ganglion-blocking drugs and indeed it may be severe enough to constitute a surgical emergency (Grant and Boyd, 1957). Roback and Beal (1953) have demonstrated that "Probanthine" by mouth in therapeutic doses produces a marked and prolonged inhibition of stomach and bowel motility. This is a particularly undesirable side-effect to encounter during the treatment of severe pancreatitis which is itself frequently complicated by persistent and troublesome ileus. It was therefore with interest that it was observed that the other ganglion-blocking agent studied - "Buscopan" - did not appear to have this disadvantage. This has been attributed to its selective action on the parasympathetic ganglia without any effect on the myo-neural junctions. Pharmacological studies

by Wick (1957) have shown that "Buscopan" counteracts pilocarpine-induced intestinal spasm but has little effect upon the intestinal spasm induced by acetylcholine which has its main point of action at the nerve endings. It would therefore be expected to have little effect upon normal muscle tone.

(iv). Conclusions:

1. When "Probanthine" or "Buscopan" were given to rats in full doses the mean serum amylase levels were lower by 26 per cent and 37 per cent respectively than the mean level in normal rats. These reductions are statistically significant.

2. Both ganglion-blocking agents prolonged the period of hyperamylasaemia following pancreatic obstruction. I believe that these drugs, by reducing the rate of pancreatic secretion, were able to delay the onset of distension destruction of the secretory epithelium.

3. Neither of these ganglion-blocking agents was capable of reducing the level of hyperamylasaemia following the production of obstructive pancreatitis. The explanation offered is that, as the hyperamylasaemia in acute pancreatic disorders is due not to any increase in total pancreatic secretion but to

the diversion of a greater part of the secretion into the interstitial tissue of the pancreas, factors affecting permeability of the gland parenchyma will therefore play a more significant part in the determination of the degree of hyperamylasaemia than the limited reductions in total pancreatic secretion which can be produced with ganglion-blocking agents.

4. There appeared to be more abdominal distension and ileus in the rats receiving "Probanthine" than among the controls. This is a recognised side-effect of the drug. "Buscopan" did not appear to have this disadvantage.

(b) Cortisone

(i) Introduction. In 1952 Stephenson, Pfeffer and Saypol reported the successful treatment with cortisone of acute haemorrhagic pancreatitis in a patient who was gravely ill and severely shocked, and since then there have been many reports of similar experiences (Hoste, 1953; Eskwith, Cacace and Sollosy, 1955; Elman, 1955; Jones, 1955; Bloodworth and Cohen, 1955; Brockis and Jones, 1956; Rodgers, Meynell, Wilson and Cooke, 1956; Kaplan, 1957b). Successes also with corticotrophin have been reported by Hume and Moore (1951), Suzman (1953) and Solem, Knutrud and Andresen (1955).

From these reports there is good evidence that cortisone and corticotrophin have been of real value in the resuscitation of severely shocked and collapsed patients. The claims by some (Hume and Moore, 1951; Kaplan, 1957 a, b) that these steroids have a specific protective action upon the pancreas when given in acute pancreatitis are less convincing and there is evidence accumulating that, especially when given over a long time, they may even facilitate the production of acute pancreatitis. Acute pancreatitis arising during treatment with cortisone or corticotrophin has been reported by Zion, Goldberg and Suzman (1955), Baar and Wolff (1957), Marczynska-Robowska (1957), Bourne and

Dawson (1958), and the view that this occurrence is not coincidental is supported by the pathological studies of Carone and Liebow (1957). Carone and Liebow made histological examinations of the pancreas taken at autopsy in 54 patients who had been under treatment with corticotrophin or adrenal steroids, and they found ductular proliferations in over half, and fat necrosis and acute pancreatitis in more than a quarter, of the organs examined. In none of these patients had there been any clinical manifestations of acute pancreatitis during life. These findings are in line with the earlier experimental work by Stumpf, Wilens and Somoza (1956) and Bencosme and Lazarus (1956), who had independently examined the histological effects of prolonged and high doses of cortisone upon the pancreas of the rabbit.

Carone and Liebow expressed the view that "during the active destructive phase of severe acute pancreatitis, it is unlikely that short courses of adrenal corticoids to combat hypotension or overwhelming toxic effects would cause appreciable further destruction. During the recovery phase these agents, by obstructing the pancreatic acini, may delay healing or cause exacerbation." A further danger in the routine use of these steroids in the treatment of acute pancreatitis is the known

occurrence of serious complications, such as peptic ulceration (Stewart, Elliott and Zollinger, 1958; Pollock, 1959).

Little appears to have been written upon the effect of these corticoids upon the serum amylase in the presence of pancreatic disease apart from the comment by Hume and Moore that "A.C.T.H. invariably caused a fall in serum amylase." I have therefore studied the effect of cortisone upon the serum amylase level in rats, normal and with experimental pancreatitis. These results are correlated with clinical and other findings.

(ii) Experiments: A. The Effect of Cortisone in the Normal Rat.

1. Method: The effect of cortisone upon the serum amylase level in 21 normal rats was studied. In 12, cortisone was given daily in a dosage of 2.5 mg. (10 mg./Kg. body weight) intramuscularly, and the rats were killed on the 5th, 7th and 12th days; in the remaining 9 rats the dosage of cortisone was doubled and the animals were killed on the 8th and 13th days.

2. Results (Table XXIII): The range of values obtained was 1,237-2,872 units/100 ml., with an arithmetic mean of 1,859, standard deviation of 381 and coefficient of variation of 20.5. When these results are compared with those found in the control series (see Appendix C.1), it will be observed that the mean value in the cortisone-treated rats of 1,859 units/100 ml. closely approximates to the control mean of 1,810, the difference being insignificant. The results in the cortisone-treated rats show a much greater scatter, the coefficient of variation being 20.5 compared with 12.5 in the controls.

B. Cortisone in Experimental Pancreatitis in the Rat.

1. Method: For the investigation into the effect of cortisone in obstructive pancreatitis, the two rat preparations previously described were used. Cortisone was injected intramuscularly in dosage of 12.5 mg./Kg. body weight daily. At the same time, control rats were subjected to duct ligation but received no cortisone. Groups of animals, including both cortisone-treated and control rats, were sacrificed at intervals of 24, 36, 48 and 72 hours after duct ligation. A general autopsy examination was made with particular attention to the integrity of the ligatures and degree of bile duct distension present, and the presence and extent of peritonitis, ileus and

TABLE XXIII. Effect of Cortisone upon Serum Amylase in Normal Rat

Dose of Cortisone	10 mg./kg. body wt. daily			20 mg./kg. body wt. daily	
No. of days	5	7	12	8	13
Serum Amylase (u/100ml)	2105 1879 1769	2238 2146 2014	1757 1572 1651 1440 1309 1237	2029 1589 1503 1580	2872 2201 2163 2160 1781
Mean	1917	2133	1503	1675	2235
No. of Rats	3	3	6	4	5

Arithmetic Mean (whole series) - 1859

Standard Deviation - 381

fat necrosis. In a proportion of cases the pancreas was removed for histological examination.

A few rats succumbed prematurely during the course of the experiments but complete studies were possible in 22 rats which had double ligature of the biliary-pancreatic duct (i.e. obstruction without biliary reflux) and cortisone, and in 25 rats with single ligature of the biliary-pancreatic duct (i.e. obstruction with biliary reflux) and cortisone.

2. Results: The results in the cortisone-treated rats are detailed in the appendix (Tables C.6, A and B) and are summarised in Table XXIV. These results along with those obtained in the controls have been plotted alongside in Figs. 12 A and B. It will be observed that at 24 and 36 hours post-ligation, the mean values in the cortisone-treated rats are about 2,000 units higher than those of the control series, but in view of the wide scatter in results this difference is not statistically significant. The highest values at 24 hours and 36 hours post-ligation were amongst the cortisone-treated animals, but these values also remain within the range of chance.

Other Findings. In these experiments there was no difference in the clinical progress, the average amount of fat

TABLE XXIV.

Ligation of Biliary-Pancreatic Duct and Cortisone

A. Without Biliary Regurgitation

	Post-ligation Serum Amylase (units/100 ml.)			
	24 hrs.	36 hrs.	48 hrs.	72 hrs.
Range	14,148-8,022	24,036-9,471	11,976-7,485	1,620-1,372
Mean	11,737	13,668	9,346	1,519
S.D.	2,518	5,071	2,342	-
No. of Rats	7	9	3	3
	B. With Biliary Regurgitation			
Range	23,748-7,246	20,330-7,645	11,701-6,270	2,532-1,878
Mean	12,242	10,886	9,272	2,246
S.D.	4,904	4,761	1,905	-
No. of Rats	10	6	6	3

Figure 13 a.

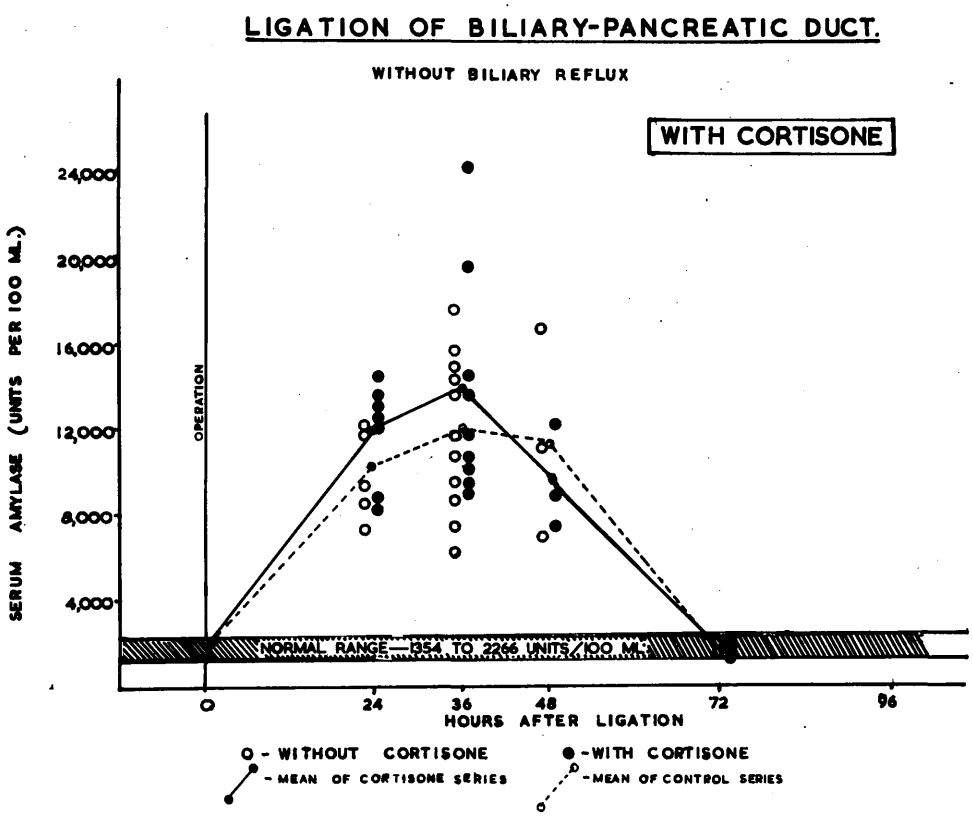
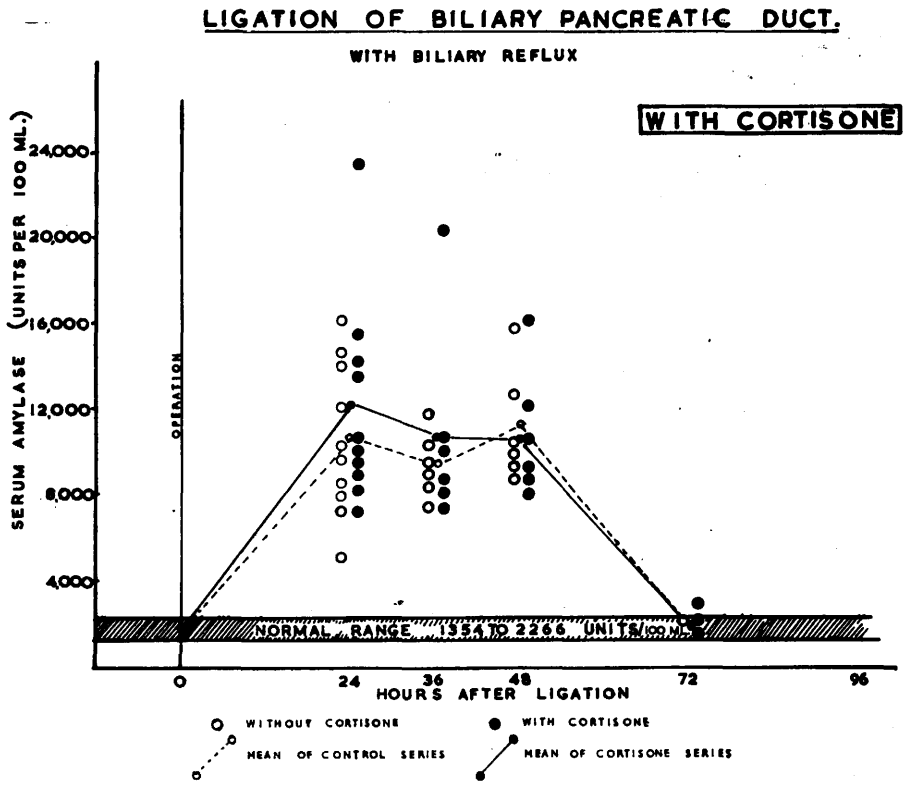

LIGATION OF BILIARY-PANCREATIC DUCT.
WITHOUT BILIARY REFLUX
WITH CORTISONE
SERUM AMYLASE (UNITS PER 100 ML.)
24,000
20,000
16,000
12,000
8,000
4,000
OPERATION
NORMAL RANGE—1354 TO 2266 UNITS/100 ML.
0
24
36
48
72
96
HOURS AFTER LIGATION
O - WITHOUT CORTISONE
● - WITH CORTISONE
- MEAN OF CORTISONE SERIES
- MEAN OF CONTROL SERIES

Figure 13 b.


LIGATION OF BILIARY PANCREATIC DUCT.
WITH BILIARY REFLUX
WITH CORTISONE
SERUM AMYLASE (UNITS PER 100 ML.)
24,000
20,000
16,000
12,000
8,000
4,000
OPERATION
NORMAL RANGE 1354 TO 2266 UNITS/100 ML.
0
24
36
48
72
96
HOURS AFTER LIGATION
WITHOUT CORTISONE
WITH CORTISONE
MEAN OF CONTROL SERIES
MEAN OF CORTISONE SERIES

necrosis present nor in the mortality rate between the cortisone-treated and the control animals. The effect of cortisone upon the rats with biliary-pancreatic duct obstruction appeared to be the same whether bile was permitted to escape into the obstructed pancreatic ducts or not.

(iii). Conclusion: From these experiments no evidence was forthcoming to show that cortisone lowered the serum amylase level or had any other beneficial effect upon the pancreas in experimentally produced obstructive pancreatitis in the rat, and no evidence was obtained to support the view that cortisone may help pancreatitis in man.

(c) Propyl-thiouracil

(i) Introduction. A new approach to the treatment of pancreatitis has been the use of propyl-thiouracil as a general anti-metabolic agent to suppress pancreatic intracellular enzyme activity. Starr (1954 and 1955), acting upon the observation that thyroid extract taken by patients immediately after an operation had been associated with haemorrhagic pancreatitis and other serious post-operative complications, tried the effect of propyl-thiouracil in pancreatitis. He formed the impression that adequate doses of propyl-thiouracil were of value in the early stages of pancreatitis.

Reid et al. (1957, 1958 a, b) have studied the matter in more detail. They found that by using very large doses of propyl-thiouracil in dogs (300 mg./dog) the serum amylase level was lowered in 15 out of 18 dogs. Of the remaining three dogs, two had their serum amylase level significantly lowered by increasing the dosage of the propyl-thiouracil. These same workers (Paulette et al., 1958), by using doses of propyl-thiouracil in the region of 1,000 mg. per dog, were able to reduce the incidence of experimentally-induced haemorrhagic pancreatitis in dogs.

Reid et al. (1957) believe that the rapid haemorrhagic necrosis in pancreatitis is due to the release of the intracellular enzymes of cell metabolism rather than to the action of trypsin or chymotrypsin. The intracellular enzymes of the pancreas (whose function, amongst many others, is the building of lipase, amylase, trypsin and trypsinogen) are capable of immense protein synthesizing activity. The pancreas has the greatest protein output per gram weight tissue of any organ or tissue in the body and as the catalytic enzyme reaction is reversible under certain circumstances (Bergman, 1953), a potential for intense proteolytic activity is also present. There is, for example, rapid autolysis of the pancreas after death. As propyl-thiouracil inhibits the uptake of oxygen in all tissues and organs (Reid and Kossa, 1954), it might in the pancreas prevent the formation of the pancreatic enzymes in the intact cells and restrain the degrading activity of the released intracellular enzymes.

It was therefore decided to feed rats with large doses of propyl-thiouracil and to study particularly its effects upon the amylase in the blood in the normal animal and after ligation of the biliary-pancreatic duct. Several rats from each group also received I^{131} and at the end of the experiment

the thyroid glands were extracted and studied by means of radio-chromatograms. By this means a comparison was made between the anti-metabolic action of propyl-thiouracil upon the thyroid and upon the pancreas. (These isotope studies were carried out by Dr. E. M. McGirr).

(ii) Method. As in the previous studies, male albino Wistar rats of 220-250 G. weight were used. The propyl-thiouracil was made up in a suspension of 25 mg. propyl-thiouracil in 0.2 ml. water with the addition of a small amount of gum acacia, and given by gastric tube.

(1) Six rats were fed with 25 mg. propyl-thiouracil (100 mg./kg. body weight) daily for five days. Blood was then aspirated by cardiac puncture for serum amylase estimation and the animals autopsied.

(2) Thirty rats had their common biliary-pancreatic duct tied. They received a single dose of propyl-thiouracil 24 hours before operation and daily after operation until sacrificed. Groups of rats were killed at 24, 36, 48, 72 and 96 hours after duct ligation.

(iii). Results: A. Effect of Intragastric Propyl-thiouracil in Normal Rat. The results of a series of six rats are given in Table XXV. It will be seen that the range of serum amylase was 1774-1273 units/100 mg. with a mean of 1358 units/100 mg. and a standard deviation of 203 units/100 mg. In normal unmedicated rats the range was 2290-1504 units with a mean of 1810 units/100 ml. and a standard deviation of 228. Thus the administration of propyl-thiouracil to rats without pancreatic duct obstruction resulted in significantly lower levels of serum amylase (t-test gives $P < 0.01$).

B. Effect of Intragastric Propyl-thiouracil and Biliary-Pancreatic Duct Obstruction. The serum amylase levels obtained are recorded in the appendix (Table C.7) and are analysed in Table XXVI and shown graphically in Fig. 14. In the presence of biliary-pancreatic duct obstruction, propyl-thiouracil did not significantly affect the pattern of the serum amylase curve found in unmediated animals, there being at no stage a significant difference between these results and the controls.

The paper chromatograms after injection of tracer doses of I^{131} showed that the intragastric propyl-thiouracil was effective in completely blocking thyroxine synthesis, particularly at the mono-iodothyrosine and di-iodo-tyrosine levels (Appendix C.8).

TABLE XXV

EFFECT OF INTRAGASTRIC PROPYL-THIOURACIL UPON SERUM AMYLASE

Serum Amylase in Six Rats on 25 mgm. Propyl-thiouracil (100 mgm./kilogm. body weight) for 5 days

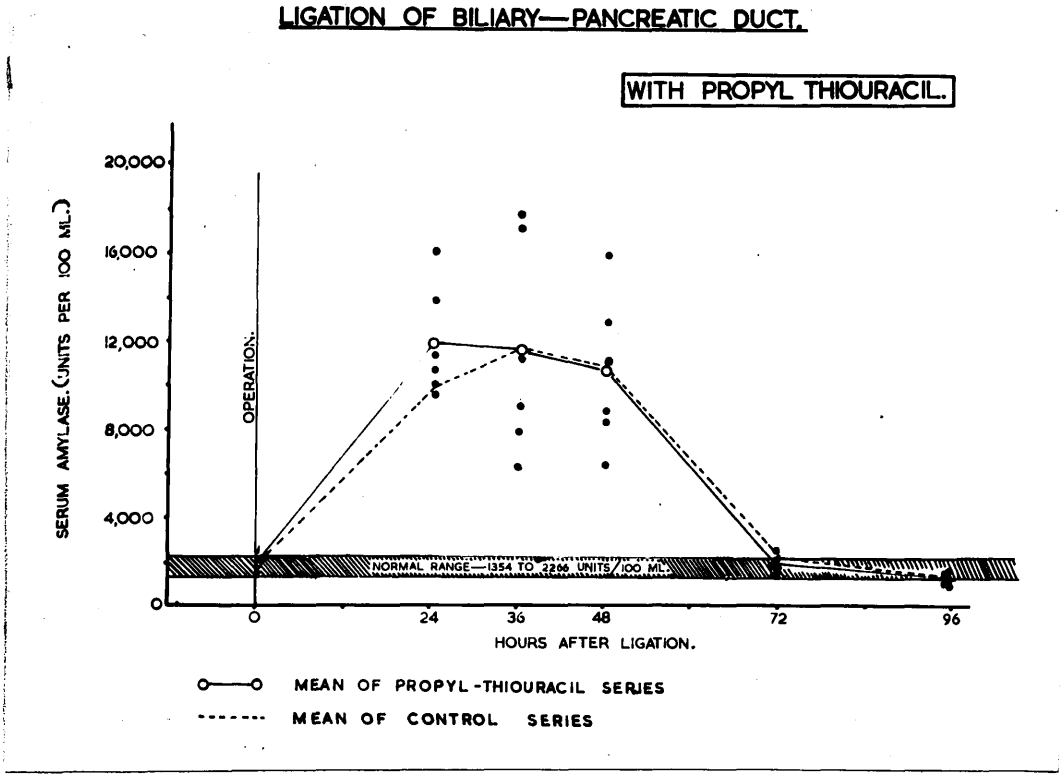
Values obtained	1774, 1494, 1425, 1399, 1386, 1273
Range	1774 - 1273
Arithmetic Mean	1358
Standard Deviation	203
A.M. ± 2 S.D.	1761 - 952

TABLE XXVI

LIGATION OF BILIARY-PANCREATIC DUCT AND PROPYL-THIOURACIL

	Post-ligation Serum Amylase (Units/100 ml.)				
	24 hrs.	36 hrs.	48 hrs.	72 hrs.	96 hrs.
Range	16,170-9,813	17,795-6,255	15,882-6,536	2,485-1,661	2,025-1,207
A.M.	12,080	11,666	10,600	2,037	1,540
S.D.	2,533	4,791	3,420	329	320
No. of Rats	6	6	6	5	5

Figure 14.


LIGATION OF BILIARY—PANCREATIC DUCT.
WITH PROPYL THIOURACIL.
SERUM AMYLASE. (UNITS PER 100 ML.)
20,000
16,000
12,000
8,000
4,000
0
OPERATION.
NORMAL RANGE—1354 TO 2266 UNITS/100 ML.
0
24
36
48
72
96
HOURS AFTER LIGATION.
MEAN OF PROPYL-THIOURACIL SERIES
MEAN OF CONTROL SERIES

(iv). Conclusions. That propyl-thiouracil lowers the serum amylase level in the normal rat is confirmed. This seems to be related to the general anti-metabolic effect rather than to any specific effect upon the pancreas.

In so far as the serum amylase level can be taken as a measure of the pancreatic enzyme concentration in the interstitial tissues, no evidence was afforded here that propyl-thiouracil is able to reduce this enzyme spill.

The dose of propyl-thiouracil given to the rats in these experiments (100 mg./kg. weight) is comparable to the very large doses given by Reid et al. (1958) to their dogs and is greatly in excess of the normal therapeutic dose of propyl-thiouracil in humans. The rats did not tolerate this dosage well and appeared toxic and ill. Reid et al. (1958) found that, though propyl-thiouracil inhibited in rats the oxygen uptake of all tissues, it also caused adrenal atrophy, inhibition of growth and bizarre cerebral manifestations. It would therefore seem likely that this drug is too toxic for clinical use and might be dangerous when used in a disease which is characterised in its acute phase by intense adrenal activity and which may prove fatal from relative adrenal insufficiency.

(d) Acetazolamide ("Diamox")

(i) Introduction. Hitherto, studies have been directed towards the reduction of enzyme secretion by chemical "vagotomy" using the ganglion-blocking agents, Probanthine and Buscopan, and by inhibition at cell level using the anti-metabolic substance propyl-thiouracil. In the normal pancreas, secretion of enzymes is small in volume compared with that of water and bicarbonate. If therefore the volume of the secretion could be significantly reduced by inhibition of the water and sodium bicarbonate fractions, then it would be expected that in the presence of duct obstruction there would be a less rapid spill of water, bicarbonate and enzymes into the interstitial tissues and therefore less pancreatic damage. Treatment along these lines might prove to be a useful contribution towards the treatment of acute obstructive pancreatitis in man.

It has been claimed (Still, Bennett and Scott, 1933) that the bicarbonate secreted in the pancreatic juice is due to carbonic anhydrase activity in the pancreatic gland and the carbonic anhydrase inhibitor acetazolamide ("Diamox") has been found to be effective in reducing the volume of pancreatic secretion in patients with pancreatic fistulae (Hollander and

Birnbaum, 1952; Papstrat and Miller, 1948; Watman, 1956). I have therefore studied the effect of this drug in experimentally-induced pancreatitis in the rat.

(ii) Method. As in the previous experiments, male albino rats (Wistar strain) of about 300 G. weight were used. In the first experiment the effect of acetazolamide in the normal rat was studied. Acetazolamide was given in dosage of 12.5 mg. b.i.d. subcutaneously (80 mg. acetazolamide/kg./day) for 48 hours, when the animals were sacrificed and a general examination as well as a serum amylase estimation was made. This dose is comparable to that stated by Janowitz (1958) to be the minimum effective dose in humans. It was found to be rather toxic in rats and in subsequent experiments the dosage was reduced to 60 mg./kg. body weight daily.

The effect of the drug in the rat with pancreatic duct obstruction was then investigated. In the first experiments the common biliary-pancreatic duct was ligated distally only (Preparation B). As however acetazolamide does not have any known inhibitory effect upon biliary secretions, it might be that a fall in the rate and volume of pancreatic secretion could be compensated by an increased flow of bile into the pancreatic ducts and moreover the mixing of bile with

concentrated enzymes in the pancreatic ducts might facilitate their permeation into the gland substance with increased tissue damage. A further series of experiments was therefore carried out in which the biliary and pancreatic duct systems were isolated by a proximal ligature around the biliary-pancreatic duct above the pancreatic ducts, as in Preparation A. In both these groups of experiments acetazolamide was given in doses equivalent to 60 mg./kg. weight.

(iii) Results: A. The Effects of Acetazolamide in Normal Rats.

Serum Amylase: The serum amylase readings are recorded in the accompanying table (Table XXVII). There is an insignificant rise from the values found in healthy rats without acetazolamide.

Other Biochemical Findings: It has already been mentioned that rats appeared toxic and ill after receiving acetazolamide in doses of 60-80 mg./kg. body weight daily for two days. Further biochemical studies were carried out in three rats that had received 60 mg./kg. acetazolamide for 48 hours and these studies showed that the animals had a marked acidosis with an average fall in the alkali reserve of 27 per cent.

TABLE XXVII

EFFECT OF ACETAZOLAMIDE UPON SERUM AMYLASE IN THE NORMAL RAT (FIVE RATS)

	Acetazolamide 12.5 mgm. b.d.	Without Drugs
Serum Amylase Range	2306-1912	2288-1504
Arithmetic Mean	2,013	1,810
Standard Deviation	164	228

There were no significant alterations in the serum potassium or sodium level. The detailed figures are given in the appendix (Table C.8).

B. Acetazolamide and Pancreatic Duct Obstruction.

Serum Amylase: The results are summarised in the accompanying Table XXVIII and illustrated in Fig. 15. Details are in the appendix (Table C.9). The readings at 24, 36 and 48 hours post-ligation are within the normal range and therefore at these times the acetazolamide has had no significant effect upon the serum amylase level. This was true with both the rat preparations used, so that the presence or absence of biliary reflux into the pancreatic tree has had no significant effect upon these results.

At 72 hours after operation the serum amylase level remained significantly raised compared with the results in the control series ($P < 0.01$) without any difference between the two experimental groups. The elevations were less than those obtained at 72 hours when either of the ganglion-blocking agents was used.

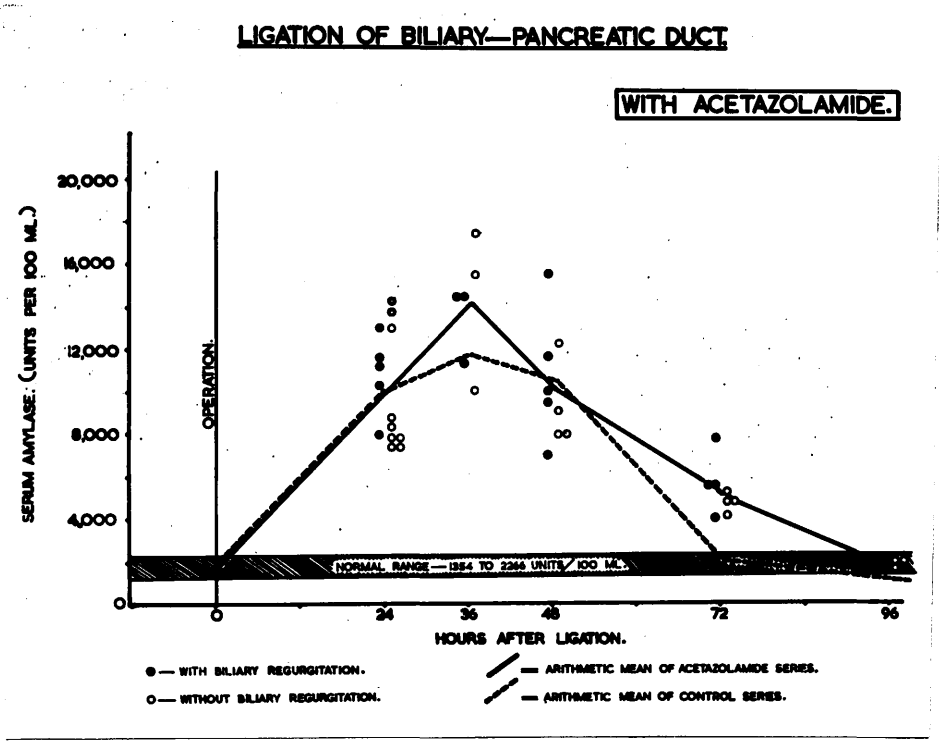
TABLE XXVIII. Ligation of Biliary-Pancreatic Duct and Acetazolamide

		Post-Ligation Serum Amylase (Units/100 ml.)			
		24 hrs.	48 hrs.	72 hrs.	96 hrs.
GROUP A	Range	13,013-10,276	15,495-7,121	7,685-4,070	2,130-1,416
	Mean	10,805	10,764	5,670	1,748
	S.D.	1,787	3,102	1,497	-
	No. of Rats Surviving	5	5	4	2
GROUP B	Range	13,365-7,642	12,234-7,951	5,350-4,213	2,020-1,753
	Mean	9,570	9,273	4,653	1,830
	S.D.	2,406	2,028	717	-
	No. of Rats Surviving	9	4	4	3

Group A: With biliary regurgitation

Group B: Without biliary regurgitation

Figure 15.


LIGATION OF BILIARY—PANCREATIC DUCT.
WITH ACETAZOLAMIDE.
SERUM AMYLASE: (UNITS PER 100 ML.)
20,000
16,000
12,000
8,000
4,000
0
OPERATION.
NORMAL RANGE — 1364 TO 2266 UNITS / 100 ML.
0
24
36
48
72
96
HOURS AFTER LIGATION.
● — WITH BILIARY REGURGITATION.
○ — WITHOUT BILIARY REGURGITATION.
— ARITHMETIC MEAN OF ACETAZOLAMIDE SERIES.
— ARITHMETIC MEAN OF CONTROL SERIES.

General Appearance and Behaviour: The rate in these experiments were more toxic and ill than the corresponding rats in the previous series of experiments and their belly fur was continually sodden with the constant diuresis. In the first group of rats that had single ligation, 8 out of 24 succumbed during the course of the experiments (33 per cent mortality). In the second group, which had double ligation of the common biliary-pancreatic duct, only 20 out of the 28 operated upon survived the required length of time (28.5 per cent mortality). Of the total sixteen deaths, nine were within the first 48 hours; three were between 48 and 72 hours; of the four rats which died after 72 hours, two were found to have ruptured ducts. These mortality rates are in excess of those obtained in previous series of experiments.

The intra-abdominal appearances showed nothing of significance. Some fat necrosis was constantly present, varying from grade + to grade + + + and the pancreas showed the general macroscopic changes already described. Peritonitis and paralytic ileus were not prominent features.

(iv) Discussion. The use of acetazolamide is based on the assumption that:

1. Reduction in the volume of pancreatic juice secreted will reduce the amount of damage to the gland and the severity of the disease in acute pancreatitis. As the enzyme fraction is volumetrically small, this would most effectively be done by blocking the secretion of water and bicarbonate.

2. Carbonic anhydrase is necessary for the secretion of bicarbonate (and water) by the pancreas.

3. The carbonic anhydrase activity in the pancreas can be blocked by giving a carbonic anhydrase inhibitor, e.g. acetazolamide, without seriously affecting other vital functions.

The role of the carbonic anhydrase in the pancreas is still obscure. Carbonic anhydrase is a zinc containing enzyme, which is present in high concentrations in the erythrocytes and the renal cortex. In the former tissue it plays a vital function in the carriage of CO_2 in the blood and its liberation in the lungs. In the kidneys its function is no less vital as it

controls diuresis and plays a part in the acid-base balance in the body. Carbonic anhydrase is also present in the gastric mucosa and Van Goor (1933, 1940) reported that it was also present in the pancreas where it catalysed the carbonic ion. Its part in the metabolism of the pancreas has been doubted by others. Davenport (1940) found that sulphanilamide failed to inhibit pancreatic secretion in any way and Tucker and Ball (1941) came to a similar conclusion. Ball et al. (1941) using radioactive carbon came to the opinion that not more than 20 per cent of the bicarbonate in the pancreatic juice was from metabolic CO_2 in the blood. However, Hollander and Birnbaum's (1952) work has supported the earlier findings of Still, Bennett and Scott (1933) that the principal source of bicarbonate ions is in the pancreatic gland and they regarded carbonic anhydrase as playing a major role in the intracellular conversion of carbon dioxide to carbonic acid and therefore bicarbonate ion.

New impetus to these investigations followed the production by Lederle Laboratories of a carbonic anhydrase inhibitor claimed to be 400 times more active than sulphonil-amide - 2 acetyl-amine 1, 3, 4 thiadiazole-5-sulfonamide ("Diamox" acetazolamide). Dreiling, Janowitz and Halpern (1955) in clinical studies found that intravenous acetazolamide

in doses above 50 mg./kg. were capable of inhibiting markedly, if not completely blocking, the total volume and total bicarbonate response of the pancreas to secretin. The basal rates of pancreatic flow and bicarbonate secretion were reduced, but there was no significant effect upon enzyme secretion. They concluded that carbonic anhydrase is necessary for bicarbonate secretion, that this secretion could be blocked by the use of a carbonic anhydrase inhibitor and that the control of volume at cellular level might have therapeutic indications.

(v) Conclusions. The evidence from my results in the rats is that with acetazolamide there has been some reduction in the intraductal secretion from the pancreas, as shown by the continued elevation of the serum amylase level at 72 hours, but there is no support for the view that the enzyme spill into the interstitial tissues has thereby been significantly reduced. The observations that these rats were more ill, were acidotic and that these experiments were accompanied by a higher mortality rate than was observed in similar operations either without a drug or with other drugs, are considered important.

The dosage of 60 mg./kg./day is several times above the usual therapeutic dose in humans but Dreiling et al. (1955) found that doses over 50 mg./acetazolamide/kg. were necessary

in the human to produce any detectable effect upon the pancreatic secretion. Those large doses are apparently well tolerated in the human without acute disease (Dreiling et al., 1955) but they could well be harmful and dangerous in acute pancreatic disease.

In severe pancreatitis, recovery may depend upon the restoration and maintenance of fluid and electrolyte balance. It would seem highly undesirable to introduce a drug which acts by upsetting the vital and delicately balanced acid-base mechanism in the body to produce an unphysiological diuresis. Moreover there is evidence that the carbonic anhydrase inhibitor is much more effective against the vital carbon dioxide transport functions of the erythrocyte and the renal cortex than upon the pancreatic secretions (Milne, 1956).

CONCLUSION

In the first part of this thesis I have studied the serum amylase in a number of healthy adults. According to the method of measurement used within this laboratory, results are within the range of 133 to 320 iodometric units with a mean of 204 iodometric units and a standard deviation of 50 iodometric units. While the level of the amylase in the blood of one individual usually remained fairly constant, divergences of as much as 65 per cent from the mean took place from day to day or from hour to hour. These fluctuations were more apparent in some persons than others, but all deviations were within the normal limits for health as given above.

The second part of this thesis describes clinical studies on 223 patients with acute abdominal disease, of which 23 had acute pancreatitis. I have been particularly concerned to determine the place of serum amylase measurement in the diagnosis of acute pancreatic disease, to compare measurements made in acute pancreatitis with those obtained in other acute abdominal conditions and to decide whether this biochemical test could with advantage permit the avoidance of surgery in the acute stage of pancreatitis.

In all of the 18 patients seen in their first attack of acute pancreatitis, the serum amylase was considerably elevated. No fewer than 16 out of the 18 cases had values of five times the upper limit of normal (1,600 iodometric units) and over; in the remaining two cases lesser elevations corresponding to about three times the upper limit of hormal (1,067 and 914 iodometric units) were observed. In the three cases seen in an acute relapse, two had serum amylase levels over 1,600 iodometric units. The third, who had a serum amylase level within the normal range, had had repeated attacks and shortly after showed further evidence of pancreatic fibrosis and insufficiency by developing diabetes. In the remaining two cases, which were examined in the late stages of an attack, insignificant values of 400 and 200 iodometric units were found.

Treatment in the acute stage was non-operative in 21 out of the 23 cases. In the other two cases laparotomies were performed as the diagnoses of perforated peptic ulcer could not be excluded on clinical grounds and a report on the serum amylase was not available. The routine therapeutic measures are detailed and a report is made of my experiences of the use of autonomic ganglion blockade with local anaesthetic and with the ganglion-blocking agents, methantheline bromide (Banthine) and

hyoscine N-butylbromide (Buscopan). The mortality rate in this series was nil. The factors contributing to the recent downward trend in the mortality rate are discussed.

In the 200 patients admitted as surgical emergencies on account of abdominal pain believed not to be pancreatic in origin, the serum amylase was found to be above the normal range in 22 (11 per cent). These elevations, ranging from 320 to 914 iodometric units, in no case exceeded five times the upper limit of the normal level.

Hyperamylasaemia was commonest among patients with acute exacerbation of biliary tract disease, among whom the incidence was 28.7 per cent, compared with an incidence in all other cases of 7.3 per cent.

It is my experience that, in the absence of defective renal function, five times the upper limit of normal represents a critical serum amylase level. Results above this, during the early stage of an acute abdominal illness, strongly support the diagnosis of acute pancreatitis; values below this level may possibly be the result of acute abdominal processes not originating in the pancreas.

The measurement of serum amylase has thus proved to be a biochemical procedure of assistance in the diagnosis of acute pancreatitis and its value for such a purpose is enhanced when its limitations are appreciated.

In the final part of this thesis I have set out to assess the ability of drugs to suppress the exocrine secretion of the pancreas during experimentally induced pancreatitis. The drugs studied are some of those at present advocated for clinical use in acute pancreatitis, namely propantheline bromide (Probanthine), hyoscine N-butyl bromide (Buscopan), cortisone, propylthiouracil and acetazolamide (Diamox).

The experimental animal used was the Wistar rat and a pancreatic disorder resembling acute interstitial pancreatitis in the human was produced by ligation of the biliary-pancreatic duct.

When rats were treated with Probanthine, Buscopan or propylthiouracil, the serum amylase levels were significantly lower than normal ($P < 0.01$). Neither cortisone nor acetazolamide had any apparent effect upon the serum amylase in the normal animal.

Experimental obstructive pancreatitis was produced in several groups of rats. In those treated with Probanthine, Buscopan or acetazolamide, enzyme secretion by the pancreas persisted for longer after duct ligation than in the control animals or in those treated with cortisone or propylthiouracil. Evidence is produced to show that this was due to a reduction in the amount of exocrine secretion accumulating in the obstructed gland. Further investigation with the ganglion-blocking drug Buscopan seemed to show that there was a reduction in the total pancreatic secretion rather than a specific inhibitory effect upon enzyme formation or secretion.

All these drugs, including those which produced a lower serum amylase level in the normal rat and apparently reduced the secretory rate in the obstructed pancreas, were ineffective in reducing at all the high level of serum amylase found in the first forty-eight hours after biliary-pancreatic duct ligation. An explanation put forward is that the rise in the serum amylase occurring after pancreatic duct obstruction or acute pancreatic disease is due, not to any increase in total pancreatic secretion, but to diversion of a greater part of the secretion into the interstitial tissues of the pancreas. Normally the amount of pancreatic secretion passing into the bloodstream is

small. In acute pancreatic disorders, however, the permeability of the gland is profoundly altered, resulting in flooding of the interstitial tissues with secretion, sufficient in amount to obscure the lesser changes consequent on partial reduction of the total pancreatic secretion. (Other experimenters have succeeded in reducing the hyperamylasaemia at this stage by using ganglion-blocking agents when there has been maximal and continued stimulation of the pancreas after duct ligation and, in these circumstances, the proportionate reduction of the pancreatic secretion by these ganglion-blocking agents is much higher). The serum amylase level will also be affected by the vascular state of the pancreas. A good blood flow would facilitate the clearance of extravasated secretion with the production of a higher enzyme content in the blood than might be found with the same degree of extravasation but a lesser blood flow.

There was no evidence that cortisone given over a short period has any specific effect upon pancreatic function.

Most of the drugs used were accompanied, in the dosage given, by undesirable or dangerous side-effects which would limit their value in clinical pancreatitis.

Drugs given to block pancreatic secretion are incomplete in their effect. The most useful, as far as could be determined by these experiments, were the ganglion-blocking agents. It would seem that hyoscine N-butyl bromide (Buscopan) was the more effective in reducing the pancreatic secretion in the rat and that it was superior to the other ganglion-blocking agent in that there were fewer side-effects, particularly upon the bowel.

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APPENDIX A

SOMOGY'S SACCHAROGENIC METHOD

for

DETERMINATION OF SERUM AMYLASE

Saccharogenic Method (Somogyi, 1938), modified by Talluto (1954)

Serum is incubated with a starch solution and the amount of reducing substance present is determined before and after the incubation. The difference gives a measure of the amylolytic activity of the serum.

Reagents

1. Starch Solution: 1.5 grams of washed starch is ground thoroughly in a mortar and then added to 70 to 80 ml. boiling water. The mortar is rinsed out a few times with a few ml. water which is added to the boiling starch solution. The solution is boiled for about one minute and then heated in a water bath for 15 to 30 minutes, keeping the mouth of the flask covered with an inverted beaker. Allow to cool and make up to 100 ml. with water.

2. Acidified Sodium Chloride Solution: Dissolve 10 grams sodium chloride in water, add 3 ml. N/10 hydrochloric acid and make up to one litre with water.

3. Sulphuric Acid, 2/3 N solution.

4. Sodium Tungstate, 10 per cent solution.

5. Somogyi's High Alkalinity Copper Reagent: Dissolve 25 grams anhydrous sodium carbonate and 25 grams Rochelle salt in about 600 ml. water, add 40 ml. normal sodium hydroxide followed by 6 grams copper sulphate dissolved in about 100 ml. water and added with constant stirring. Then add 5 grams potassium iodate and shake well. When dissolved, make to one litre and mix.

6. Sulphuric Acid, 5 N.

7. N/200 Sodium Thiosulphate: Prepare freshly for use from stock. N/10 solution.

8. Starch Solution: As indicated.

Technique

Pipette 5 ml. starch solution and 2 ml. sodium chloride solution into each of three test tubes. Place two of these in a water bath at 40°C. for a few minutes to warm up and then to one add 1 ml. serum (test), and to the other 1 ml. water (water blank). Then incubate for thirty minutes. For the serum blank add 1 ml. serum to the third tube followed immediately by 1 ml.

2/3 N sulphuric acid and 1 ml. sodium tungstate solution. At the end of the half hour's digestion, remove from the bath and add, with shaking, 1 ml. each of these same solutions. Stand for a few minutes then centrifuge. Filter the supernatant fluids through small filter papers.

To each of the supernatant fluids add 5 ml. alkaline copper solution and heat in a bath of boiling water for twenty minutes, plugging the tubes lightly with cotton wool. Cool under the running water. Add 1 ml. 5 N sulphuric acid and titrate with N/200 sodium thiosulphate.. Since there is excess starch present, a blue colour is formed immediately on acidification, so that as the end-point is approached it is necessary to shake vigorously after each addition of thiosulphate to prevent overshooting of the end-point.

Calculation

The content of reducing substance in the three titrations, expressed as glucose, is read from the table on the following page and the amylase calculated as follows:

Serum amylase (units/100 ml.) = Mg. glucose/100 ml. test - mg. glucose/100 ml. serum - mg. glucose/100 ml. water blank.

For values over 300 units per 100 ml., dilute serum with 0.9 per cent saline and repeat the test.

Glucose per 100 Cc. of Solution Corresponding to Titration Values When 5 Cc. of 1:10 Solution and 5 Cc. of High Alkalinity Copper Reagent Are Heated in Water Bath for 20 Minutes

0.005 N thiosulfate	0.005 N sodium or thiosulfate									
	0	0.1 cc.	0.2 cc.	0.3 cc.	0.4 cc.	0.5 cc.	0.6 cc.	0.7 cc.	0.8 cc.	0.9 cc.
	Glucose in 100 cc. of blood or plasma									
cc.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg*
0					18	22	26	29	32	35
1	39	42	45	48	52	55	58	61	65	68
2	71	74	78	81	84	87	90	93	96	99
3	102	106	109	112	115	118	121	124	127	130
4	133	137	140	143	146	149	152	155	158	161
5	164	168	171	174	177	180	183	186	189	192
6	195	199	202	205	208	211	214	217	220	223
7	226	229	232	235	238	241	244	247	250	253
8	256	260	263	266	269	272	275	278	281	284
9	287	290	293	296	299	302	305	308	311	314
10	317	321	324	327	330	333	336	339	342	345
11	348	352	355	358	361	364	367	370	373	376
12	379	383	386	389	392	395	398	401	404	407
13	410	414	417	420	423	426	429	432	435	438
14	441	445	448	451	454	457	460	463	466	469
15	472	475	478	481	484	487	490	493	496	499
16	502	506	509	512	515	518				

Table reproduced from M. Somogyi's article, 'Micromethods of Estimation of Diastase', J. biol. Chem. 125:403.

APPENDIX B

SUMMARY OF CASES

OF

PANCREATITIS

Case 1. A. A., female aged 66 years.

Provisional Diagnosis: Choledocholithiasis.

Clinical Features: Admitted with pain in right hypochondrium and intermittent vomiting. Had previous attack a year ago. Jaundiced. Murphy positive.

Blood: W.B.C. - 18,600/cu.mm.
Serum amylase over 1,600 units.

Urine: Bile present.

X-ray: Poorly functioning gall bladder.
No stones seen.

Treatment: Conservative. Followed up for two months without recurrence.

Final Diagnosis: Acute cholecystitis; pancreatitis.

Case 2. C. A., female aged 69 years.

Provisional Diagnosis: ? Coronary thrombosis ? pancreatitis.

Clinical Features: While in ward with suspected cholelithiasis, sudden collapse with praecordial pain, pallor and cyanosis. E.C.G. normal.

Blood:	Serum amylase (2 hours after onset) over 4,000 units.
X-ray:	Gallstones present.
Treatment:	Conservative (pethidine). No follow up.
Final Diagnosis:	Cholelithiasis; acute pancreatitis.

Case 3. M. J., female aged 56 years.

Provisional Diagnosis:	Stone in common bile duct.
Clinical Features:	Pain in right hypochondrium and vomiting for 5 days. Several attacks over the past month.
Blood:	Jaundiced - Van den Bergh 12.5 mgm. bilirubin/100 ml. Serum amylase 2,133 units.
X-ray:	Cholecystogram negative but operative cholangiogram showed small stone at lower end of common bile duct.
Treatment:	Immediate - conservative. Choledochostomy and cholecystectomy after 16 days. No follow up.
Final Diagnosis:	Choledocholithiasis; acute pancreatitis.

Case 4. W. C., male aged 68 years.

Provisional Diagnosis:	Cholelithiasis.
Clinical Features:	Constant pain in right hypochondrium with some vomiting for 5 days. Slight jaundice.
Blood:	Serum amylase: 1,600 units (on admission) 533 units (48 hours later)
Urine:	Trace of bile.
Electrocardiogram:	Posterior myocardial insufficiency.
X-ray:	Functioning gall bladder. No stones seen.
Treatment:	Conservative.
Final Diagnosis:	? Cholecystitis; pancreatitis.

Case 5. M. A., female aged 27 years.

Provisional Diagnosis: Acute pancreatitis.

Clinical Features: Admitted with nausea, retching and severe pain in epigastrium and left loin for 48 hours. Jaundiced, acutely distressed with shallow breathing. No bowel sounds heard.

Blood: W.B.C. 12,000/cu.mm.
Serum amylase 1,600 units.

Urine: Bile + + +; trace albumen; no sugar.

X-ray: Chest negative. Scout film of abdomen showed segmental ileus jejunum.

Treatment: Continual gastric suction and parenteral fluids. Coeliac plexus block (15 ml. 1% xylocaine right and left) - no benefit.

Penicillin and streptomycin (2 weeks) - Aureomycin - Erythromycin.

Pethidine 100 mgm. S.O.S.

Progress:

First Week: Fairly rapid relief of the acute symptoms but persistent distension with nausea and vomiting. Serum amylase within normal limits by fourth day but jaundice tended to deepen.

Second Week: Continued to have troublesome ileus. Bowel sounds first audible on seventh day and gastric suction and intravenous therapy stopped on twelfth day.

Third Week: General improvement in condition with temperature settling and jaundice getting less. Palpable swelling in epigastrium and left hypochondrium. ? Swollen pancreas or developing pseudo-cyst.

Fourth Week: Onset of drug-resistent staphylococcal interstitial pneumonia affecting left side and then spreading to right. Retroperitoneal swelling in left hypochondrium displacing the stomach downwards and forwards. Some ileus of splenic flexure and small bowel.

Laparotomy: On opening the abdomen there was widespread fat necrosis with calcification. The upper abdominal organs were matted together and the pancreas was greatly enlarged and hard. The liver was distinctly enlarged and almost black in colour with icterus. The gall bladder contained stones. No subphrenic abscess was present. The gall bladder was emptied of stones (over 600) and drained. Abdomen closed.

Further Progress: Following operation the patient continued critically ill with an irregular pyrexia (up to 103°F.) for eight weeks, with marked tachycardia and irregular respiration. Clinically and radiologically there was a slowly progressive bilateral basal pneumonia with small left pleural effusion. This was not appreciably influenced by aureomycin or erythromycin but the progression seemed to be halted and reversed by repeated blood transfusions. Dismissed for convalescence after twelve weeks. Seen three years later having remained well without jaundice or symptoms.

Case 6. M. S., female aged 55 years.

Provisional Diagnosis:	Pancreatitis.
Clinical Features:	Admitted with sudden severe and constant epigastric pain of six hours' duration. Patient shocked (B.P. 100/50), pale and frequently vomiting small amounts of bile-stained fluid.
Blood:	Serum amylase 1,600 units (on admission) 2,133 units (after 24 hours).
X-ray:	Cholecystogram showed no stones.
Progress:	Improved with conservative treatment (pethidine, continual gastric suction and penicillin and streptomycin) but abdominal pain persisted. After 48 hours a coeliac plexus block with 1% xylocaine gave permanent alleviation. Subsequently occasional nausea and flatulence but no pain.

Case 7. M. G., female aged 58 years.

Provisional Diagnosis:	Cholelithiasis and acute cholecystitis.
Clinical Features:	Admitted with pain in right hypochondrium and vomiting for several hours. Has had similar attacks over the past four years, precipitated by taking potatoes or fatty foods. Grossly obese woman, retching and in obvious distress. Not jaundiced.
Blood:	Serum amylase 2,560 units. 72 hours later - 400 units.
X-rays:	Biliary tract not investigated as patient transferred elsewhere.
Progress:	Settled in 3-4 hours with conservative treatment.

Case 8. E. McC., female aged 78 years.

Provisional Diagnosis:	Acute pancreatitis.
Clinical Features:	Abdominal discomfort for a week with sudden onset of severe pain and nausea for a few hours. Obese, distressed old lady sitting propped up in bed with dyspnoea. Not jaundiced. Retching without vomit. Upper abdomen distended and tympanitic.
Blood:	Serum amylase 2,133 units, falling to 200 units after 48 hours. W.B.C. 22,450 cells/cu.mm.

Urine:	No bile, albumin or sugar present.
X-rays:	Gas-filled coils of small intestine visible in right lumbar region.
Treatment:	Settled rapidly with conservative treatment. No follow-up.

Case 9. B. G., female aged 78 years.

Provisional Diagnosis:	? Cholelithiasis; ? pancreatitis.
Clinical Features:	Admitted with pain, nausea and vomiting for three days. Pain radiated from right hypochrondrium into left flank.
Blood:	Serum amylase 2,667 units.
Urine:	No albumin, bile or sugar present.
X-ray:	Cholecystogram showed multiple stones in poorly functioning gall bladder.
Treatment:	Conservative. Coeliac plexus block with 1% xylocaine.
Progress:	Recovery uneventful. No follow-up.
Final Diagnosis:	Cholelithiasis; acute pancreatitis.

Case 10. J. R., male aged 54 years.

Provisional Diagnosis:	Obstructive jaundice.
Clinical Features:	Admitted with epigastric pain for five days. On the third day pain passed through to the back and was accompanied by nausea and vomiting. Jaundiced on admission (Van den Bergh 7.5 units/bilirubin/100 ml.) with slight tenderness in right hypochondrium and a palpable liver.
Blood:	Serum amylase "over 400 units" and 3,200 units on repeat.
Urine:	Bile present. No glycosuria.
Treatment:	Conservative. Laparotomy on re-admission for relapse.
Progress:	Discharged "well" on the nineteenth day but had a relapse with colicky abdominal pain and jaundice about three weeks later. At laparotomy there was a cholecystitis but no cholelithiasis. There was a subsiding pancreatitis with minute foci of fat necrosis. Patient has continued well since dismissal.
Final Diagnosis:	Acute non-calculus cholecystitis; pancreatitis.

Case 11. M. K., female aged 50 years.

Provisional Diagnosis:	Acute pancreatitis.
Clinical Features:	Sudden onset of epigastric pain radiating to hypochondrium and back, of a few hours' duration. Vomiting followed. Patient was pale, shocked and cyanosed on admission. Writhing and retching with only slight upper abdominal guarding. B.P. 120/80. History of addiction to alcoholic excess.
Blood:	Serum amylase 1,600 units (on admission) 400 units (after 24 hours) 200 units (after 48 hours).
Urine:	No bile or glycosuria.
X-ray:	Cholecystogram (after two weeks) - negative.
Treatment:	Conservative.
Progress:	Uneventful.

Case 12. B. T., female aged 70 years.

Provisional Diagnosis:	Acute pancreatitis; cholelithiasis.
Clinical Features:	Admitted with severe abdominal pain and retching of 12 hours' duration. Elderly obese woman, looking ill, with a distended and generally tender abdomen. Had been in hospital four months previously with diagnosis of "diverticulitis" (no serum amylase measurements).
Blood:	Serum amylase 2,133 units (following morning).
Urine:	No sugar, bile or albumin. Urinary diastase - over 400 Wohlgemuth Units.
Treatment:	Pethidine, intravenous fluids, penicillin and streptomycin.
Progress:	On third day passed rectally a large number of small facetted gallstones. Dismissed home on ninth day. Since then has had periodic attacks of abdominal pain with nausea and vomiting.
Final Diagnosis:	Cholelithiasis; acute pancreatitis.

Case 13. J. C., female aged 59 years.

Provisional Diagnosis:	Obstructive jaundice due to choledocholithiasis.
Clinical Features:	Intermittent pain in upper abdomen radiating into back for three weeks, with constant severe pain for twelve hours. Jaundiced. Palpable gall bladder.
Blood:	Serum amylase over 1,600 units.
Urine:	Bile present. No albumin or sugar.
X-ray:	Cholecystogram showed laminated biliary calculi in gall bladder. Dye in hepatic and bile ducts, which were slightly distended.
Treatment:	Settled with conservative treatment.
Progress:	Continued to have recurrent attacks of pain. Had cholecystectomy for cholecystitis and cholelithiasis 15 months later. Operative cholangiogram showed no evidence of stones in the common bile duct.
Final Diagnosis:	Acute cholecystitis and cholelithiasis; acute pancreatitis.

Case 14. E. F., female aged 20 years.

Provisional Diagnosis:	? Acute pancreatitis.
Clinical Features:	Complained of lower abdominal pain with vomiting for 48 hours. When admitted was in severe abdominal pain with some tenderness and guarding in upper abdomen. No jaundice.
Blood:	Serum amylase 3,200 units.
Urine:	Trace of bile. No sugar or albumin.
X-rays:	Cholecystogram (later) - negative.
Treatment:	Conservative (intravenous fluids, gastric suction and pethidine). Settled rapidly.
Progress:	No further attacks in short follow-up period.
Final Diagnosis:	Acute pancreatitis.

Case 15: J. C., male aged 53 years.

Provisional Diagnosis: ? Perforated peptic ulcer.

Clinical Features: Admitted on 7.1.58 with severe epigastric pain, retching but no vomiting, of twelve hours' duration. No history of previous dyspepsia, abdominal pain or jaundice. Short (height 5 ft. 4 in.), stout (weight 18 stones 2 pounds) man with grey appearance, sweating profusely. Guarding of upper abdomen.

Laparotomy: No gas or free fluid in abdomen. Stomach, duodenum and gall bladder normal. Pancreas thickened. No fat necrosis.

Blood: Serum amylase on admission 1,600 units; second day 320 units.

X-rays: Barium meal negative. Cholecystogram (tetra and biligrafin) negative.

Electrocardiogram: Appearances non-specific and suggested myocardial impairment.

Progress: Settled with conservative treatment and dismissed home.

Subsequent Progress: 28.1.58 - first relapse. Severe abdominal pain. Distressed and shocked. Serum amylase 1,600 units. Settled with conservative treatment.

26.5.58 - second relapse. Abdominal pain and vomiting for several hours. Serum amylase 2,133 units; w.b.c. 27,500. Appeared to respond well to Buscopan tabs. 2 four-hourly

17.6.58 - third relapse. Recurrent pain. Had stopped taking Buscopan.

30.6.58 - fourth relapse. Attended another hospital with ? peptic ulcer but no ulcer found.

October, 1958 - On Buscopan. No further attacks.

Case 16. B. B., female aged 63 years.

Provisional Diagnosis: Cholelithiasis with biliary colic.

Clinical Features: Severe abdominal pain and nausea for a few hours. Looked ill with very severe pain. B.P. 200/95. No jaundice.

Blood: Serum amylase 1,067 units on admission;
267 units after 48 hrs;
178 units on 10th day.

Electrocardiogram: Posterior myocardial ischaemia.

X-ray: Functioning gall bladder with multiple non-opaque calculi. No definite dilation of common bile duct.

Treatment: Conservative.

Progress: Recurrent attacks for 48 hours then recovery. No subsequent attacks.

Final Diagnosis: Cholelithiasis; acute pancreatitis.

Case 17. M. McL., female aged 26 years.

Provisional Diagnosis: Perforated peptic ulcer.

Clinical Features: Admitted with epigastric pain going through to the back and a little vomiting for 48 hours. Had tenderness and rigidity upper abdomen and has had intermittend epigastric pain following childbirth four months previously.

Laparotomy: There was swelling and oedema of pancreas with bloodstained exudate and fat necrosis in omentum, parietal peritoneum and transverse colon. Gall bladder and ducts normal.

Blood: Serum amylase (day after admission) 914 units.

Progress: Postoperative course uneventful. Had attack of pain with obstructive jaundice nine months later. Re-admitted with acute relapse 16 months after first attack. Pain and vomiting for eight hours. Serum amylase 2,667 units; 1,600 units (24 hours); 178 units (72 hours).

Final Diagnosis: Acute pancreatitis with acute relapses.

Case 18. J. S., male aged 32 years.

Provisional Diagnosis: Acute pancreatitis.

Clinical Features: Sudden severe epigastric pain with nausea. Has had several similar attacks in past year. B.P. 150/75.

Blood: Serum amylase 4,571 units on admission; 1,470 units after 24 hrs.

X-ray: Barium meal showed slight duodenal cap irritability without evidence of ulceration. Some radio-opaque gall stones seen in gall bladder.

Treatment: Immediate - conservative.
Cholecystectomy six weeks later.

Cholecystectomy: A small thick-walled gall bladder filled with stones was removed. No abnormality of stomach or duodenum or gross evidence of pancreatic disease. Operative cholecystogram showed normal biliary tracts without demonstrable obstruction to the flow of the contrast medium into the duodenal loop.

Progress: Convalescence complicated by a pneumonia due to Friedlander's bacillus but chest was clinically and radiologically clear on dismissal. No recurrence of abdominal pain in subsequent four months.

Case 19. C. S., female aged 68 years.

Provisional Diagnosis:	Acute relapsing pancreatitis (diagnosed elsewhere and referred).
Clinical Features:	Recurrent attacks of acute abdominal pain over two years, lasting several hours at a time.
Blood:	Serum amylase 8,000 units (when in medical ward). Liver function and blood urea normal.
Urine:	No bile, sugar or albumin.
X-rays:	Barium meal and enema negative. Cholecystogram showed a non-functioning gall bladder.
Treatment:	Conservative, with low fat diet. Laparotomy refused.
Final Diagnosis:	? Cholecystitis; acute relapsing pancreatitis.

Case 20. D. C., male aged 66 years.

Provisional Diagnosis:	Acute relapsing pancreatitis. (Pancreatitis diagnosed in 1953).
Clinical Features:	Admitted with abdominal pain and vomiting for 12 hours. Has had periodic attacks over the past four years.

1953 - laparotomy. Acute pancreatitis found. Biliary tract normal and no other abnormality found.

1956 - cholecystectomy. No gallstones.

Blood: Serum amylase over 1,600 units.

Treatment: Conservative.

Final Diagnosis: Acute relapsing pancreatitis.

Case 21. E. C., female aged 68 years.

Provisional Diagnosis: Relapsing pancreatitis.

Clinical Features: Admitted with upper abdominal pain and vomiting for three days. No jaundice. At laparotomy one year before, cholelithiasis and subsiding pancreatitis found. Cholecystectomy performed.

Blood: Serum amylase 200 units.

Urine: No sugar.

Treatment: Conservative.

Progress: Periodic attacks of pain. Subsequently developed diabetes.

Final Diagnosis: Relapsing pancreatitis.

Case 22. M. A., female aged 49 years.

Provisional Diagnosis: Cholecystitis.

Clinical Features: Admitted to another unit with pain in right hypochondrium, abdominal rigidity and guarding. Transferred when acute symptoms had subsided.

Blood: Serum amylase (14th day of illness) 400 units.

Laparotomy: Pancreas nodular and firm with areas of fat necrosis. No disease of stomach, duodenum or gall bladder.

Final Diagnosis: Subsiding pancreatitis.

Case 23. S. F., female aged 49 years.

Provisional Diagnosis: Cholelithiasis with cholecystitis.

Clinical Features: Obese woman with attacks of pain and vomiting after food for two weeks. Not jaundiced.

Blood: Serum amylase 178 units.

Progress: Condition settled rapidly and patient irregularly dismissed herself. Recurrent attack one week later and referred back to Unit after acute symptoms had settled. Serum amylase 200 units.

Laparotomy: Large gall bladder filled with gallstones. Area of pancreatitis about two inches in diameter in head of pancreas. No stones in biliary ducts. Cholecystectomy.

Final Diagnosis: Cholelithiasis; subsiding pancreatitis.

RAT EXPERIMENTS

APPENDIX C

PROTOCOLS

OF

RAT EXPERIMENTS

C.1

Serum Amylase in Albino Rats (Wistar Strain)
(25 rats : Wt. 250-350 Gm.)

Readings: (Somogyi Units/100 ml.)

2,288; 2,278; 2,172; 2,008; 2,129; 1,987; 1,901;
1,896; 1,859; 1,847; 1,825; 1,798; 1,693; 1,690;
1,679; 1,682; 1,669; 1,667; 1,648; 1,640; 1,634;
1,605; 1,568; 1,540; 1,504.

Range: 2,288 - 1,504 units/100 ml.

Mean: 1,812 units/100 ml.

Standard Deviation: 228 units/100 ml.

Coefficient of Variation: 12.6 per cent.

C.2

Serum Amylase in Obstructive Pancreatitis (without biliary reflux)

Time after Ligation	Serum Amylase Readings (Units/100 ml.)	Mean	No. of Rats
24 hrs.	12,110; 11,945; 9,627; 8,592; 7,654	10,000	5
36 hrs.	17,704; 15,761; 14,271; 14,163; 13,167; 11,073; 10,467; 9,748; 8,852; 7,289; 6,182	11,700	11
48 hrs.	16,439; 11,052; 6,670	11,400	3
72 hrs.	2,343	-	1

C.3

Serum Amylase in Obstructive Pancreatitis (with biliary reflux)

Time after Ligation	Serum Amylase Readings (Units/100 ml.)	Mean	No. of Rats
24 hrs.	16,236; 14,820; 14,771; 14,479; 12,271; 11,479; 10,083; 10,065; 8,413; 7,945; 6,967; 4,876	11,000	12
36 hrs.	18,492; 13,891; 13,605; 12,314; 11,955; 10,770; 10,942; 9,286; 9,120; 8,536; 7,945	11,530	11
48 hrs.	15,789; 12,285; 10,445; 9,483; 8,993; 9,344; 7,486; 5,252	9,890	8
66 hrs.	1,985; 2,213; 2,808	2,335	3
72 hrs.	2,358; 1,958; 1,288; 1,240	1,710	4
96 hrs.	1,824; 1,380; 1,145; 784	1,283	4

C.4

Serum Amylase in Obstructive Pancreatitis and Probanthine

Time after Ligation	Serum Amylase Readings (Units/100 ml.)	Mean	No. of Rats
24 hrs.	17,245; 15,357; 8,551	13,717	3
36 hrs.	18,907; 16,804; 15,661; 13,571; 12,855; 10,076	14,646	6
48 hrs.	11,529; 10,929; 6,533	9,664	3
72 hrs.	19,613; 12,227; 10,734; 7,789; 7,346; 6,958; 5,527; 5,520	9,468	8
96 hrs.	2,194; 1,730; 1,591; 1,581; 1,201; 901	1,560	6

C.5

Serum Amylase in Obstructive Pancreatitis and Buscopan

Time after Ligation	Serum Amylase Readings (Units/100 ml.)	Mean	No. of Rats
24 hrs.	15,892; 14,848; 14,531; 14,389; 10,558; 9,841	13,343	6
36 hrs.	14,727; 13,124; 11,995; 8,546; 7,957	11,269	5
48 hrs.	12,430; 9,750; 9,299; 8,882; 8,080; 6,960; 6,945	8,906	7
72 hrs.	10,242; 10,042; 8,536; 6,810; 6,373; 5,626	7,938	6
96 hrs.	2,008; 1,989; 1,328	1,775	3

C.6

Serum Amylase in Obstructive Pancreatitis and Cortisone (12.5 mgm./kilogm. body weight)

A. Without Biliary Reflux

Time after Ligation	Serum Amylase Readings (mgm. per cent)	Mean	No. of Rats
24 hrs.	14,148; 13,348; 12,992; 12,845; 12,573; 8,229; 8,022	11,737	7
36 hrs.	24,036; 19,487; 14,859; 13,560; 11,676; 10,417; 9,908; 9,595; 9,471	13,668	9
48 hrs.	11,976; 8,576; 7,485	9,346	3
72 hrs.	1,620; 1,565; 1,372	1,519	3
	B. With Biliary Reflux		
24 hrs.	23,748; 15,657; 14,282; 13,854; 10,598; 10,432; 8,576; 9,457; 8,567; 7,246	12,242	10
36 hrs.	20,330; 10,598; 10,023; 8,457; 8,260; 7,645	10,886	6
48 hrs.	11,701; 10,789; 9,598; 8,720; 8,557; 6,270	9,272	6
72 hrs.	2,532; 2,329; 1,878	2,246	3

C.7.

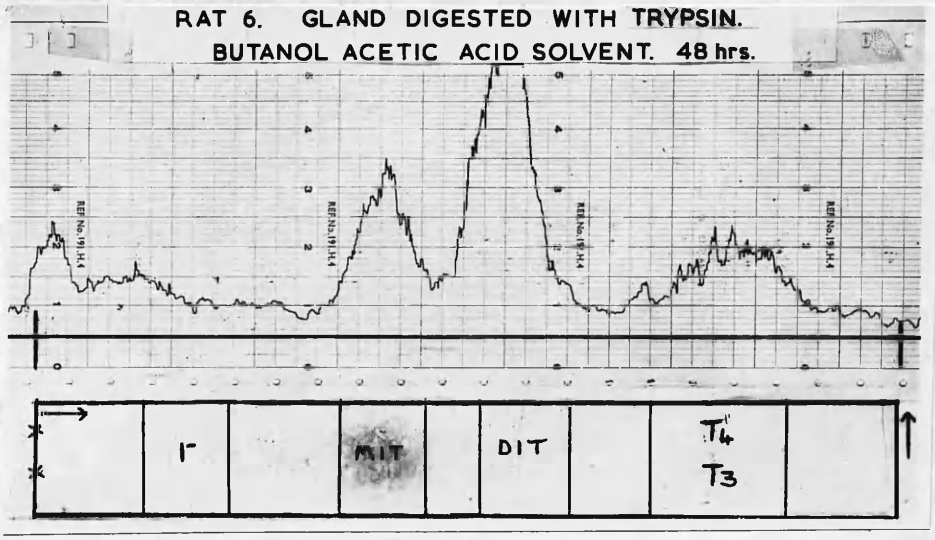
Serum Amylase in Obstructive Pancreatitis and Propylthiouracil (100 mg./kilogm. body weight)

Time after Ligation	Serum Amylase Readings (mg. per cent)	Mean	No. of Rats
24 hrs.	16,170; 14,063; 11,590; 10,852; 9,964; 9,813	12,080	6
36 hrs.	17,795; 17,345; 11,452; 9,095 8,053; 6,255.	11,666	6
48 hrs.	15,882; 12,784; 11,272; 8,633; 8,401; 6,536.	10,600	6
72 hrs.	2,485; 2,176; 2,090; 1,773; 1,661.	2,037	5
96 hrs.	2,025; 1,671; 1,465; 1,333; 1,207.	1,540	5

Radio-chromatograms Showing Effect of Propylthiouracil on Thyroxine Synthesis.

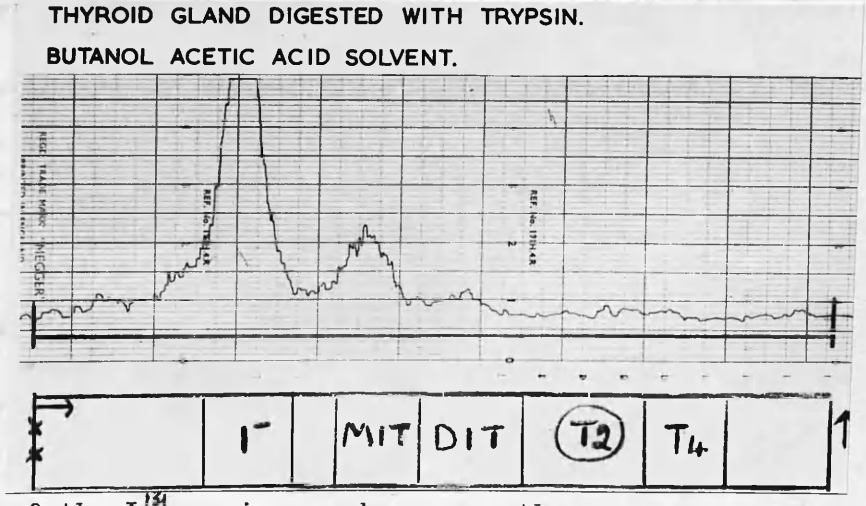
The iodine-containing protein fractions have been separated and identified by paper chromatography (lower part); the upper tracing is of the radioactivity present in these protein fractions. 50 microcuries I^{131} injected 24 hours before sacrifice.

A. No propylthiouracil.



The I^{131} present in the gland has been synthesised into mono--iodotyrosine (D.I.T.), di-iodotyrosine (M.I.T.), and thyroxine (T4,T3).

B. Propylthiouracil (25 mg. for three days).



Most of the I^{131} remains, and no more than a trace of thyroxine has been formed.

C.9.

Blood Chemistry in Rats Receiving Acetazolamide.

1. Plasma Alkali Reserve (Vols. per cent)

(a) With Acetazolamide	Readings	47.9; 44.9; 48.9; 40.3; 50.8.
	Range	40.3 - 50.8
	Average	46.5
(b) Controls	Readings	56.3; 60.0; 61.0; 57.2; 62.3
	Range	56.3 - 62.3
	Average	59.4

Average percentage fall in alkali reserve - 21.7 %

2. Serum Sodium (mEq/l.)

(a) With Acetazolamide (3 rats) Readings 145; 140; 143.

(b) Controls (3 rats) Readings 145; 145; 149.

No significant change

3. Serum Potassium (mEq/l.)

(a) With Acetazolamide (3 rats) Readings 4.9; 4.8; 5.1;

(b) Controls (3 rats) Readings 4.9; 4.3; 4.1.

No significant change

C.10

Serum Amylase in Obstructive Pancreatitis and Acetazolamide

A. With Biliary Reflux

Time after Ligation	Serum Amylase Readings (mg. per cent)	Mean	No. of Rats
24 hrs.	13,013; 11,380; 11,220; 10,276; 8,136	10,805	5
48 hrs.	15,495; 11,673; 9,915; 9,614 7,121.	10,764	5
72 hrs.	7,685; 5,548; 5,370; 4,070.	5,670	4
96 hrs.	2,130; 1,416.	1,748	2

B. Without Biliary Reflux

24 hrs.	13,365; 12,730; 11,975; 8,755; 8,410; 7,807; 7,670; 7,642; 7,686.	9,570	9
48 hrs.	12,234; 8,961; 7,946; 7,951.	9,273	4
72 hrs.	5,350; 4,822; 4,218; 4,213.	4,653	3

APPENDIX D

The Significance of Hyperamylasaemia in Surgical Conditions: Serum Amylase Investigations in 181 Patients with Acute Abdominal Symptoms.

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